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PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF DISEASES RELATED TO NEUROTROPHINES

FIELD OF THE INVENTION

The present invention refers to pharmaceutical compositions comprising 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I), their dimers of general formula (II) or (III) hereinafter reported, or mixtures thereof, useful in the treatment of pathologies in which the neurotrophine functions, particularly of Nerve Growth Factor (NGF), are altered.

STATE OF THE ART

Numerous proteins and polypeptidic factors regulate cell growth and/or survival. The first of such factors which was identified and functionally characterised is NGF. Later on, other proteins belonging to the same NGF family were identified that exert their activity on different populations of nervous cells. All these proteins are collectively referred to as "neurotrophins".

NGF, upon interaction with specific surface receptors, prevents neuronal cell death during embryonal development and throughout adult life. NGF administration was proven advantageous in pathological conditions, such as degenerative and ischaemic disorders of Central Nervous System (CNS), spinal lesions, and toxicity of excitory amino acids. In fact, together with other neurotrophic factors, NGF promotes neuronal regeneration and supports neuronal functions.

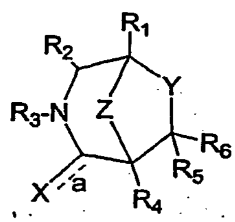
Therapeutic uses of NGF have been limited by its poor ability to get across the blood-brain-barrier, partly due to the molecular size of the native factor. Thus, the development of non-peptidic compounds able to specifically mimic the activities of the natural ligand is a useful approach to obviate such limitations. Relevant examples of such compounds are: a) phorbol esters, that mimic NGF presumably by modifying PKCc activity; b) ganglioside and other unrelated lipidic compounds, that promote neuritic outgrowth from dorsal root ganglia, or other sympathetic, neurones; c) Triap (1,1,3-triciano-2-ammino-1-propene), a small compound able to support survival and induce neuritic growth in PC12 cells. In all of the above cases, activity of molecules is not mediated by interactions with NGF receptors. Development of new non-peptidic compounds able to interact with specific

receptors, thus behaving as agonists or antagonists, of human neurotrophins is of utmost importance, since they may be used as drugs for treatment of disorders related to a defective or excessive activity of neurotrophins.

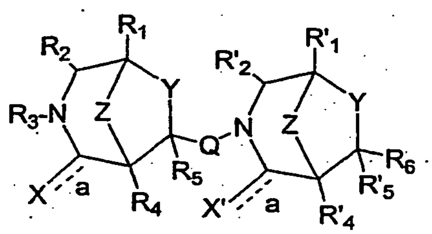
SUMMARY OF THE INVENTION

Now, the Applicants have unexpectedly found that 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I) and their dimers of general formula (II) and (III) as reported hereinafter, are active as agonists of human neurotrophines, therefore they are useful for preparation of pharmaceutical compositions for the treatment of diseases in which the neurotrophine functions, particularly the NGF functions, are involved in defect.

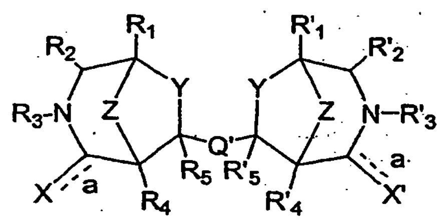
It is therefore subject of the present invention a pharmaceutical composition comprising as the active principle at least one among the 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I), or their dimers of general formula (II) and (III), or mixtures thereof:



I



II



III

wherein:

R_1 and R'_1, equal or different between each other, are selected from the group consisting of H, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, cycloalkyl, aryl, heterocycle, arylC_{1-8}alkyl; heterocycleC_{1-8}alkyl, RR'N-C_{1-8}alkyl, RR'N-aryl, FmocNR'-aryl, BocNR'-aryl, CBzNR'-aryl, RO-aryl, R(O)C-aryl, RO(O)C-aryl, RR'N(O)C-aryl; FmocNR'-C_{1-8}alkyl, BocNR'-C_{1-8}alkyl, CbzNR'-C_{1-8}alkyl, FmocNR'-C_{1-8}aryl, BocNR'-C_{1-8}aryl and CbzNR'-C_{1-8} aryl,

R_2 and R'_2, equal or different between each other, are selected from the group consisting of H, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, cycloalkyl, aryl, arylC_{1-8}alkyl, heterocycleC_{1-8}alkyl, aminoC_{1-8}alkyl, aminoaryl, C_{1-8}alkyloxyaryl, hydroxyaryl, hydroxyC_{1-8}alkyl, carboxyC_{1-8}alkyl, methyloxycarbonylC_{1-8}alkyl, carboxyaryl, carboalkyloxyaryl, alkylcarbamoylaryl and -(side chains of amino acids), or

R_1 and R_2, taken together, and R_1' and R_2', taken together, are C_{1-4}alkyl, C_{2-4} alkenyl, cycloalkyl or benzofused cycloalkyl, to form a bridge of 3, 4, 5, 6 terms,

R_3 and R_3' are selected from the group consisting of H, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, cycloalkyl, aryl, arylC_{1-8}alkyl, heterocycleC_{1-8}alkyl, RR'NC_{1-8}alkyl, RR'Naryl, RO-C_{1-8}alkyl, RO(O)C-C_{1-8}alkyl, R(O)C-C_{1-8}alkyl, RC(O)O-C_{1-8}alkyl, RC(O)N(R)C_{1-8}alkyl, RO-aryl, RO(O)C-aryl, R(O)C-aryl RC(O)O-aryl, RC(O)N(R)aryl, -CH(amino acid side-chain)CO_2R, -CH(amino acid side-chain)C(O)NR, -CH(CO_2R)- amino acid side-chain, CH(CONRR')- amino acid side-chain, Fmoc, Boc and Cbz,

R_4, R'_4 R_5, and R'_5, equal or different amongst each other, are selected from the group consisting of H, C_{1-8}alkyl, C_{2-8}alchenyl, C_{2-8}alchinyl, cycloalkyl, aryl, heterocycle, arylC_{1-8}alkyl and heterocycleC_{1-8}alkyl,

R_6 is selected from the group consisting of H, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, cycloalkyl, aryl, arylC_{1-8}alkyl, heterocycle, heterocycleC_{1-8}alkyl; -C(O)R, -C(O)OR, -C(O)NRR', CH_2OR, CH_2NRR', -C(O)NH-CH(amino acid side-chain)C(O)OR, CH_2NR-Fmoc, CH_2NR-Boc and CH_2NR-CBz,

R and R', equal or different between each other, are selected from the group consisting of H, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, cycloalkyl, aryl, heterocycle, arylC_{1-8}alkyl; heterocycleC_{1-8}alkyl; protecting group, -C(O)CH-(amino acid side-chain)-NHT, -NH-CH(amino acid side-chain)COOT and -CH(amino acid side-chain)COOT,

where T is selected from between H and C_{1-8}alkyl;

X and X', equal or different between each other, are selected from between O and S, when *a* is a double bond, or

X and X' are both H, when *a* is a single bond,

Y and Z, equal or different from each other, are selected from the group consisting of O, S, SO, SO_2 and N-R, wherein R is as above defined;

Q is selected from the group consisting of C=O, CH_2, CO-NH-CH (amino acid side-chain)-CO, CONR$(CH_2)_n$CO, CONR-C_{2-8}alkenyl-CO C(O)O$(CH_2)_n$CO, CH_2OC(O)$(CH_2)_n$CO, and CH_2NRC(O)$(CH_2)_n$CO, wherein n is comprised between 2 and 6, and R is as above defined,

Q' is selected from the group consisting of $C(O)OCH_2$, $C(O)NRCH_2$, $CH_2OC(O)$, $CH_2NRC(O)$, $CONR(CH_2)_nNRCO$, $CONR\text{-}C_{2\text{-}8}$alkenyl-NRCO, $C(O)O(CH_2)_nNRCO$, $CONR(CH_2)_nOC(O)$, $CH_2OC(O)(CH_2)_nOC(O)CH_2$, $CH_2NRC(O)(CH_2)_nNRC(O)CH_2$, $CH_2OC(O)(CH_2)_nNRC(O)CH_2$, $CH_2NRC(O)(CH_2)_nOC(O)CH_2$, $CH_2NR(CH_2)_nNRCH_2$, $CH_2O(CH_2)_nOCH_2$, $CH_2O(CH_2)_nNRCH_2$, and $CH_2NR(CH_2)_nOCH_2$, wherein n is comprised between 2 and 6, and R is as above defined,

and where the groups alkyl, alkenyl, alkynyl, cycloalkyl, aryl and the heterocyclic groups above reported, are possibly substituted.

Further subject of the invention are the novel 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I) and their dimers of general formula (II) and (III) above reported.

Further subject of the invention is the use of 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I) and their dimers of general formula (II) and (III) above reported for the preparation of pharmaceutical compositions useful for the treatment of:

i) neurodegenerative disorders of the Central Nervous System, such as Alzheimer Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Huntington disease, neuropathies, neural damage caused by hypoxia, ischaemia, or trauma, inducing apoptosis of nervous cells;

ii) acquired immunodeficiency diseases related reduced bioavailability of NGF, such as immunodeficiency of ageing;

iii) diseases in which stimulation of neoangiogenesis turns out to be advantageous, such as myocardial infarction, stroke, or peripheral vasculopathies;

iv) certain pathologies of the eye, such keratitis of diverse aetiology, glaucoma, degenerative or inflammatory conditions of the retina.

Further subject of the invention is the use of 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I), their dimers of general formula (II) or (III) above reported, and mixtures thereof, for the preparation of culture and storage media useful for conservation of explanted corneas destined to transplantation, and the use for promoting *in vivo*, *in vitro*, or *ex vivo* growth and/or survival of neural cells.

Subject of the invention is also the use of 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I), their dimers of general formula (II) or (III) above reported, and mixtures thereof, labelled with suitable reagents (contrast agents, radioisotopes, fluorescent agents etc.), and processed with any procedure useful for medical imaging purposes, for the imaging analysis of tissues and organs containing neurotrophine receptors, either *in vitro* or *in vivo*, in particular for monitoring the use and efficacy of drugs, as well as for the diagnosis of mammal diseases in which the neurothrophine receptors are involved.

The characteristic and advantages of the pharmaceutical compositions according the invention will be in detail reported in the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effect of present compounds on PC12 cell survival in serum-free conditions, by using hrNGF as internal standard according to paragraph "Biological Activity" hereinafter reported. Results were expressed as survival induced by compounds/spontaneous survival $\cdot$100 for the compounds indicated on x axis.

Figure 2 shows the effect of present compounds on proliferative activity of PC3 cell line, in serum-free conditions, evaluated by using hrNGF as internal standard according to paragraph "Biological Activity". Results are expressed in terms of stimulation index, i.e. as ratio between ^{3}H-thymidine incorporation (mean $\pm$ SD) of stimulated cultures and ^{3}H-thymidine incorporation of non stimulated cultures, for the compounds indicated on x axis.

Figure 3 illustrates the ability of present compounds (I), (II) and (III) to induce the VGF production by PC12 cells, evaluated as hereinafter described in paragraph "Biological Activity" in comparison with hrNGF. The control is 68 Kda VGF.

Figures 4a and 4b show the ability of present compounds to displace the ^{125}I-NGF binding to PC12 cells, by a displacement curve obtained by analysing the resultant cell bound radioactivity in the presence of the present compounds or in the presence of hrNGF with adequate software (Graphit 4) according to paragraph "Biological Activity".

Figure 4a shows the displacement curve obtained with the present compound 9 used as competitor. The analysis of data revealed a Kd of 165 nM $\pm$ 0.05.

Figure 4b shows the displacement curve obtained by using hrNGF as competitor. The analysis of data revealed a Kd of 114 pM ± 0.01.

Figure 5 shows the ability of the present compounds 272, 325, 9 and 91 to induce Trk-A autophosphorylation, by using hrNGF as internal standard according to paragraph "Biological Activity".

Figure 6 shows the results obtained for the present compounds 9 and 325 and for the combination of the same two compounds, in a PC12 survival assay in serum-free condition, according to paragraph "Biological Activity". The results were expressed as survival induced by compounds/spontaneous survival • 100.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention by the expression "amino acid side chain" it is meant the side chain moieties of the natural occurring L or D amino acids or of the rare or non naturally occurring amino acids.

If it is not otherwise specified, the terms alkyl, alkenyl, alkynyl, aryl, arylalkyl, cycloalkyl and heterocycle, as used in the present invention, should be meant as follows:

- C_{1-8} alkyl, C_{2-8} alkenyl and C_{2-8} alkynyl relate to linear or branched alkyl radicals, having only single bonds, at least one double bond, at least one triple bond respectively. Examples of alkylic groups according the present invention include but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl, heptyl, octyl. Examples of alkenyl groups, according to the present invention, include but are not limited to ethenyl, propenyl, 1-butenyl, cis-2-butenyl, trans-2-butenyl, 2-methyl-1-propenyl, 1-pentenyl, cis-2-pentenyl, trans-2-pentenyl, 2-methyl-2-butenyl. Examples of alkynyl groups according to the present invention include, but are not limited to, ethynyl, propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, 3-methyl-1-butynyl;

- by the term "cycloalkyl" a ring containing carbon atom is meant, generally having from 3 to 8 members, and preferably from 5 to 6 members. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, norbornanyl, canphanyl, adamantanyl;

- the term “aryl” indicates a group containing one or more unsaturated rings, each ring having from 5 to 8 members, preferably 5 or 6 members. Examples of aryl groups include, but are not limited to phenyl, biphenyl and naphtyl;
- the term “heterocycle” relates to saturated or aromatic heterocycles containing one or more heteroatoms, and preferably one or more N atoms. Examples of heterocycles include, but are not limited to pyridine, imidazole, pyrrole, indole, triazoles, pyrrolidine, pyperidine;
- the term “arylalkyl” indicates a group having an alkyl and an aryl substituent as above defined. As example, arylalkyl includes but is not limited to ethylphenyl, isobutylphenyl, benzyl, ethylbenzyl, propylbenzyl, isopropylbenzyl, butylbenzyl, isobutylbenzyl, cycloexylbenzyl, stirenyl and biphenyl.

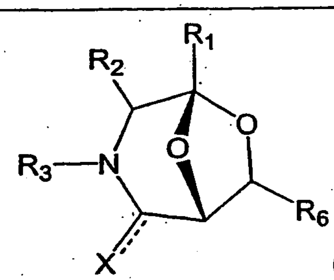
In the present invention the groups fluorenylmethoxycarbonyl, t-butyloxycarbonyl, carboxybenzyl, benzyl, phenyl and acetyl are indicated using the common terms Fmoc, Boc, Cbz, Bn, Ph and Ac respectively.

Preferred are the present compounds of formula (I), (II) and (III) wherein Z is O.

According to the present invention the alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heterocyclic groups may be substituted with one or more moieties, and preferably one or two moieties chosen from the group consisting of halogen, cyano, nitro, amino, hydroxy, carboxylic acid, carbonyl and C_{1-6} alkyl. The term “halogen” relates to fluorine, chlorine, bromine and iodine.

Among the compounds of general formula (I), (II) and (III) according the invention, the specific compounds reported in the following Tables 1-4 resulted of particular interest for their agonist activity against neurotrophines, and in particular of human NGF; and thus they are the compounds preferably used for the preparation of the pharmaceutical compositions according to the invention.

Table 1



(I)

Compound	X	R_1	R_2	R_3	R_6
1	O	H	H	$PhCH_2$	(*R*) $-CO_2Me$
2	O	H	H	$PhCH_2$	(*S*) $-CO_2Me$
3	O	H	H	$PhCH_2$	(*R*) $-CON(CH_2)_5$
4	O	H	H	$PhCH_2$	(*R*) $-CON(CH_2)_4$
5	O	H	(*S*) -Me	$PhCH_2$	(*R*) $-CO_2Me$
6	O	H	(*S*) -Me	$PhCH_2$	(*S*) $-CO_2Me$
7	O	H	(*R*) -Me	$PhCH_2$	(*R*) $-CO_2Me$
8	O	H	(*R*) -Me	$PhCH_2$	(*S*) $-CO_2Me$
9	O	H	(*R*) $-CH_2Ph$	$PhCH_2$	(*S*) $-CO_2Me$
10	O	H	(*R*) $-CH_2Ph$	$PhCH_2$	(*R*) $-CO_2Me$
11	O	H	(*S*) $-CH_2Ph$	$PhCH_2$	(*S*) $-CO_2Me$
12	O	H	(*S*) $-CH_2Ph$	$PhCH_2$	(*R*) $-CO_2Me$
13	O	H	(*S*)$-CH_2OBn$	$PhCH_2$	(*R*) $-CO_2Me$
14	O	H	(*S*)$-CH_2OBn$	$PhCH_2$	(*S*) $-CO_2Me$
15	O	H	(*R*)$-CH_2OBn$	$PhCH_2$	(*R*) $-CO_2Me$
16	O	H	(*R*)$-CH_2OBn$	$PhCH_2$	(*S*) $-CO_2Me$
17	O	H	(*S*)$-CH_2OH$	$PhCH_2$	(*R*) $-CO_2Me$
18	O	H	(*S*)$-CH_2OH$	$PhCH_2$	(*S*) $-CO_2Me$
19	O	H	(*R*)$-CH_2OH$	$PhCH_2$	(*R*) $-CO_2Me$
20	O	H	(*R*)$-CH_2OH$	$PhCH_2$	(*S*) $-CO_2Me$
21	O	H	$=CH_2$	$PhCH_2$	(*R*) $-CO_2Me$
22	O	H	$=CH_2$	$PhCH_2$	(*S*) $-CO_2Me$
23	O	H	(*R*)$-CH_2OH$	$PhCH_2$	(*S*) $-CO_2Me$
24	S	H	H	$PhCH_2$	(*R*) $-CO_2Me$
25	S	H	H	$PhCH_2$	(*R*) $-CONH(CH_2)_2NH_2$
26	S	H	H	$PhCH_2$	(*R*) $-CONH(CH_2)_2OH$
27	O	Ph	H	$PhCH_2$	(*R*) $-CO_2Me$
28	O	Ph	H	$PhCH_2$	(*S*) $-CO_2Me$
29	O	Ph	H	$CH(Ph)_2$	(*R*) $-CO_2Me$
30	O	Ph	H	$CH(Ph)_2$	(*S*) $-CO_2Me$

31	O	NO_2-Ph	H	Ph	(*S*)-CO_2Me
32	H	H	H	H	(*R*) -CO_2H
33	H	H	H	H	(*S*) -CO_2H
34	H	H	H	H	(*R*) -CO_2Me
35	H	H	H	H	(*S*) -CO_2Me
36	H	H	H	$PhCH_2$	(*R*) -CO_2H
37	H	H	H	$PhCH_2$	(*S*) -CO_2H
38	H	H	H	Fmoc	(*R*) -CO_2H
39	H	H	H	Fmoc	(*S*) -CO_2H
40	H	H	H	$PhCH_2$	(*R*) -CO_2Me
41	H	H	H	$PhCH_2$	(*S*) -CO_2Me
42	H	H	H	Boc	(*R*) -CO_2Me
43	H	H	H	Boc	(*S*) -CO_2Me
44	H	H	H	Fmoc	(*R*) -CO_2Me
45	H	H	H	Fmoc	(*S*) -CO_2Me
46	H	H	H	H	(*R*) -CONHMe
47	H	H	H	H	(*S*) -CONHMe
48	H	H	H	Ac	(*R*) -CONHMe
49	H	H	H	Ac	(*S*) -CONHMe
50	H	H	H	$PhCH_2$	(*R*) -CONHMe
51	H	H	H	$PhCH_2$	(*S*) -CONHMe
52	H	H	H	Fmoc	(*R*) -CONHMe
53	H	H	H	Fmoc	(*S*) -CONHMe
54	H	H	H	$PhCH_2$	(*R*) -$CON(CH_2)_5$
55	H	H	H	$PhCH_2$	(R) -CONHcyclohexyl
56	H	H	H	$PhCH_2$	(*R*) -$CON(CH_2)_4$
57	H	H	H	$PhCH_2$	(*R*)-$CONH(CH_2)_2OH$
58	H	H	H	H	(*R*) -CH_2OH
59	H	H	H	H	(*S*) -CH_2OH
60	H	H	H	Fmoc	(*S*) -CH_2OH

61	H	H	H	Fmoc	(*R*) $-CH_2OH$
62	H	H	H	Boc	(*R*) $-CH_2OH$
63	H	H	H	Boc	(*S*) $-CH_2OH$
64	H	H	H	$PhCH_2$	(*R*) $-CH_2OH$
65	H	H	H	$PhCH_2$	(*S*) $-CH_2OH$
66	H	H	(*S*) $-CH_2OBn$	$PhCH_2$	(*R*) $-CO_2Me$
67	H	H	(*S*) $-CH_2OBn$	$PhCH_2$	(*S*) $-CO_2Me$
68	H	H	(*R*) $-CH_2OBn$	$PhCH_2$	(*R*) $-CO_2Me$
69	H	H	(*R*) $-CH_2OBn$	$PhCH_2$	(*S*) $-CO_2Me$
70	H	H	(*S*) $-CH_2OBn$	$PhCH_2$	(*R*) $-CH_2OH$
71	H	H	(*S*) $-CH_2OBn$	$PhCH_2$	(*S*) $-CH_2OH$
72	H	H	(*R*) $-CH_2OBn$	$PhCH_2$	(*R*) $-CH_2OH$
73	H	H	(*R*) $-CH_2OBn$	$PhCH_2$	(*S*) $-CH_2OH$
75	H	H	(*S*) -COOH	Fmoc	(*R*) $-CO_2Me$
76	H	H	(*S*) -COOH	Fmoc	(*S*) $-CO_2Me$
77	H	H	(*R*) -COOH	Fmoc	(*R*) $-CO_2Me$
78	H	H	(*R*) -COOH	Fmoc	(*S*) $-CO_2Me$
79	H	H	(*S*) $-CH_2OBn$	Fmoc	(*R*) $-CO_2Me$
80	H	H	(*S*) $-CH_2OBn$	Fmoc	(*S*) $-CO_2Me$
81	H	H	(*R*) $-CH_2OBn$	Fmoc	(*R*) $-CO_2Me$
82	H	H	(*R*) $-CH_2OBn$	Fmoc	(*S*) $-CO_2Me$
83	H	H	(*S*) $-CH_2OBn$	H	(*R*) $-CO_2Me$
84	H	H	(*S*) $-CH_2OBn$	H	(*S*) $-CO_2Me$
85	H	H	(*R*) $-CH_2OBn$	H	(*R*) $-CO_2Me$
86	H	H	(*R*) $-CH_2OBn$	H	(*S*) $-CO_2Me$
87	H	H	(*S*) $-CH_2OH$	H	(*R*) $-CO_2Me$
88	H	H	(*S*) $-CH_2OH$	H	(*S*) $-CO_2Me$
89	H	H	(*R*) $-CH_2OH$	H	(*R*) $-CO_2Me$
90	H	H	(*R*) $-CH_2OH$	H	(*S*) $-CO_2Me$
91	H	H	(*S*) $-CH_2OH$	Fmoc	(*R*) $-CO_2Me$
92	H	H	(*S*) $-CH_2OH$	Fmoc	(*S*) $-CO_2Me$

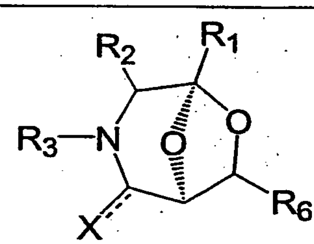
93	H	H	(*R*) -CH_2OH	Fmoc	(*R*) -CO_2Me
94	H	H	(*R*) -CH_2OH	Fmoc	(*S*) -CO_2Me
95	H	H	(*S*) -CH_2OH	Fmoc	(*R*) -CO_2Me
96	H	H	(*S*) -CH_2OH	Fmoc	(*S*) -CO_2Me
97	H	H	(*R*) -CH_2OH	Fmoc	(*R*) -CO_2Me
98	H	H	(*R*) -CH_2OH	Fmoc	(*S*) -CO_2Me
99	H	H	(*S*) -CH_2OH	$PhCH_2$	(*S*) -CO_2Me
100	H	H	(*R*) -CH_2OH	$PhCH_2$	(*R*) -CO_2Me
101	H	H	(*R*) -CH_2OH	$PhCH_2$	(*R*) -CO_2Me
102	H	H	(*R*) -CH_2OH	$PhCH_2$	(*S*) -CO_2Me
103	H	H	(*S*) -CH_2OH	Fmoc	(*R*) -CH_2OH
104	H	H	(*S*) -CH_2OH	Fmoc	(*S*) -CH_2OH
105	H	H	(*R*) -CH_2OH	Fmoc	(*R*) -CH_2OH
106	H	H	(*R*) -CH_2OH	Fmoc	(*S*) -CH_2OH
107	H	H	(*S*) -CH_2OH	$PhCH_2$	(*R*) -CH_2OH
108	H	H	(*S*) -CH_2OH	$PhCH_2$	(*S*) -CH_2OH
109	H	H	(*R*) -CH_2OH	$PhCH_2$	(*R*) -CH_2OH
110	H	H	(*R*) -CH_2OH	$PhCH_2$	(*S*) -CH_2OH
111	H	H	=CH_2	$PhCH_2$	(*R*) -CO_2Me
112	H	H	=CH_2	$PhCH_2$	(*S*) -CO_2Me
113	H	H	=CH_2	$PhCH_2$	(*R*) -CH_2OH
114	H	H	=CH_2	$PhCH_2$	(*S*) -CH_2OH
115	H	H	(*S*)-$CH_2CH(Me)_2$	Fmoc	(*R*) -CH_2OH
116	H	H	(*S*)-$CH_2CH(Me)_2$	$PhCH_2$	(*S*) -CH_2OH
117	H	H	(*S*)-$CH_2CH(Me)_2$	H	(*R*) -CH_2OH
118	H	Ph	H	H	(*R*) -CO_2Me
119	H	Ph	H	Fmoc	(*R*) -CO_2Me
120	H	Ph	H	$PhCH_2$	(*R*) -CO_2Me
121	H	Ph	H	$CH(Ph)_2$	(*R*) -CO_2Me
122	H	Ph	H	H	(*S*) -CO_2Me
123	H	Ph	H	Fmoc	(*S*) -CO_2Me

124	H	Ph	H	$PhCH_2$	(*S*) $-CO_2Me$
125	H	Ph	H	$CH(Ph)_2$	(*S*) $-CO_2Me$
126	H	p-NH_2-C_6H_4	H	Ph	(S)-COOMe
127	H	p-NH_2-C_6H_4	H	Ph	(S)-COOH
128	H	p-NH_2- C_6H_4	H	Ph	(S)-$CONHCH_2CO_2Me$
129	H	p-NH-(Asp(O^tBu)-NH_2) C_6H_4	H	Ph	(S)-CO_2Me
~~130~~	~~H~~	~~p-NH-~~ (Asp(O^tBu)N H_2)-C_6H_4	~~H~~	~~Ph~~	~~(S)-CO_2H~~
131	H	p-NH-(Asp(O^tBu)-NH_2) C_6H_4	H	Ph	(S)-CONH-Lys(NHBoc)-OMe
132	H	p-NH-(Asp(OH)-NH_2)-C_6H_4	H	Ph	(S)-CONH-Lys-OMe
133	H	p-NO_2-C_6H_4	H	Ph	(S)-COOH
134	H	p-NO_2- C_6H_4	H	Ph	(S)-COOMe
135	H	p-NO_2- C_6H_4	H	Ph	(S)-$CONHCH_2CO_2Me$
136	H	Ph	H	H	(*R*) $-CH_2OH$
137	H	Ph	H	Fmoc	(*R*) $-CH_2OH$
138	H	Ph	H	$PhCH_2$	(*R*) $-CH_2OH$
139	H	Ph	H	$CH(Ph)_2$	(*R*) $-CH_2OH$
140	H	Ph	H	H	(*S*) $-CH_2OH$
141	H	Ph	H	Fmoc	(*S*) $-CH_2OH$
142	H	Ph	H	$PhCH_2$	(*S*) $-CH_2OH$
143	H	Ph	H	$CH(Ph)_2$	(*S*) $-CH_2OH$
144	H	H	(*S*) -Me	Fmoc	(*R*) $-CO_2H$
145	H	H	(*S*) -Me	Fmoc	(*S*) $-CO_2H$
146	H	H	(*R*) -Me	Fmoc	(*R*) $-CO_2H$

147	H	H	(*R*) -Me	Fmoc	(*S*) -CO_2H
148	H	H	(*S*) -Me	Fmoc	(*R*) -CO_2Me
149	H	H	(*S*) -Me	Fmoc	(*S*) -CO_2Me
150	H	H	(*R*) -Me	Fmoc	(*R*) -CO_2Me
151	H	H	(*R*) -Me	Fmoc	(*S*) -CO_2Me
152	H	H	(*S*) -Me	$PhCH_2$	(*R*) -CO_2Me
153	H	H	(*S*) -Me	$PhCH_2$	(*S*) -CO_2Me
154	H	H	(*R*) -Me	$PhCH_2$	(*R*) -CO_2Me
155	H	H	(*R*) -Me	$PhCH_2$	(*S*) -CO_2Me
156	H	H	(*S*) -Me	Fmoc	(*R*) -CH_2OH
157	H	H	(*S*) -Me	Fmoc	(*S*) -CH_2OH
158	H	H	(*R*) -Me	Fmoc	(*R*) -CH_2OH
159	H	H	(*R*) -Me	Fmoc	(*S*) -CH_2OH
160	H	H	(*S*) -Me	$PhCH_2$	(*R*) -CH_2OH
161	H	H	(*S*) -Me	$PhCH_2$	(*S*) -CH_2OH
162	H	H	(*R*) -Me	$PhCH_2$	(*R*) -CH_2OH
163	H	H	(*R*) -Me	$PhCH_2$	(*S*) -CH_2OH
164	H	H	(*S*) -$PhCH_2$	Fmoc	(*R*) -CO_2H
165	H	H	(*S*) -$PhCH_2$	Fmoc	(*S*) -CO_2H
166	H	H	(*R*) -$PhCH_2$	Fmoc	(*R*) -CO_2H
167	H	H	(*R*) -$PhCH_2$	Fmoc	(*S*) -CO_2H
168	H	H	(*S*) -$PhCH_2$	Fmoc	(*R*) -CO_2Me
169	H	H	(*S*) -$PhCH_2$	Fmoc	(*S*) -CO_2Me
170	H	H	(*R*) -$PhCH_2$	Fmoc	(*R*) -CO_2Me
171	H	H	(*R*) -$PhCH_2$	Fmoc	(*S*) -CO_2Me
172	H	H	(*S*) -$PhCH_2$	$PhCH_2$	(*R*) -CO_2Me
173	H	H	(*S*) -$PhCH_2$	$PhCH_2$	(*S*) -CO_2Me
174	H	H	(*R*) -$PhCH_2$	$PhCH_2$	(*R*) -CO_2Me
175	H	H	(*R*) -$PhCH_2$	$PhCH_2$	(*S*) -CO_2Me
176	H	H	(*R*) -$PhCH_2$	H	(*R*) -CO_2Me
177	H	H	(*R*) -$PhCH_2$	H	(*S*) -CO_2Me

178	H	H	(*S*) -$PhCH_2$	H	(*R*) -CO_2Me
179	H	H	(*S*) -$PhCH_2$	H	(*S*) -CO_2Me
180	H	H	(*S*) -$PhCH_2$	Fmoc	(*R*) -CH_2OH
181	H	H	(*S*) -$PhCH_2$	Fmoc	(*S*) -CH_2OH
182	H	H	(*R*) -$PhCH_2$	Fmoc	(*R*) -CH_2OH
183	H	H	(*R*) -$PhCH_2$	Fmoc	(*S*) -CH_2OH
184	H	H	(*S*) -$PhCH_2$	$PhCH_2$	(*R*) -CH_2OH
185	H	H	(*S*) -$PhCH_2$	$PhCH_2$	(*S*) -CH_2OH
186	H	H	(*R*) -$PhCH_2$	$PhCH_2$	(*R*) -CH_2OH
187	H	H	(*R*) -$PhCH_2$	$PhCH_2$	(*S*) -CH_2OH
188	H	H	(S)-$PhCH_2$	$PhCH_2$	(R)-COOH
189	O	p-NO_2Ph	H	Ph	$CONH(CH_2)_6NH_2$

Table 2



(I)

Compound	X	R_1	R_2	R_3	R_6
190	O	H	H	$PhCH_2$	(*R*) -CO_2Me
191	O	H	H	$PhCH_2$	(S) -CO_2Me
192	O	H	(*S*) -Me	$PhCH_2$	(*R*) -CO_2Me
193	O	H	(*S*) -Me	$PhCH_2$	(*S*) -CO_2Me
194	O	H	(*R*) -Me	$PhCH_2$	(*R*) -CO_2Me
195	O	H	(*R*) -Me	$PhCH_2$	(*S*) -CO_2Me
196	O	H	(*S*) -$PhCH_2$	$PhCH_2$	(*R*) -CO_2Me
197	O	H	(*S*) -$PhCH_2$	$PhCH_2$	(*S*) -CO_2Me
198	O	H	(*R*) -$PhCH_2$	$PhCH_2$	(*R*) -CO_2Me
199	O	H	(*R*) -$PhCH_2$	$PhCH_2$	(*S*) -CO_2Me
200	O	H	(*S*) -$CH_2CH(Me)_2$	$PhCH_2$	(*R*) -CO_2Me

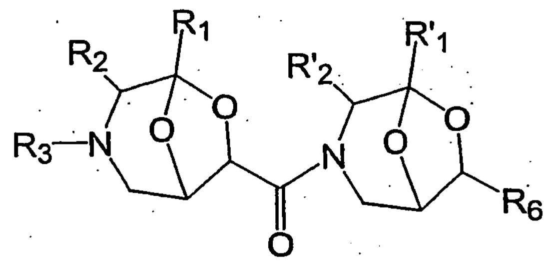
201	O	H	(*S*) -$CH_2CH(Me)_2$	$PhCH_2$	(*S*) -CO_2Me
202	O	H	(*R*) -$CH_2CH(Me)_2$	$PhCH_2$	(*R*) -CO_2Me
203	O	H	(*R*) -$CH_2CH(Me)_2$	$PhCH_2$	(*S*) -CO_2Me
204	O	H	H	$PhCH_2$	(*R*) -CONHMe
205	O	H	H	$PhCH_2$	(*S*) -CONHMe
206	O	H	(*S*) -Me	$PhCH_2$	(*R*) -CONHMe
207	O	H	(*S*) -Me	$PhCH_2$	(*S*) -CONHMe
208	O	H	(*R*) -Me	$PhCH_2$	(*R*) -CONHMe
209	O	H	(*R*) -Me	$PhCH_2$	(*S*) -CONHMe
210	O	H	(*S*) -$PhCH_2$	$PhCH_2$	(*R*) -CONHMe
211	O	H	(*S*) -$PhCH_2$	$PhCH_2$	(*S*) -CONHMe
212	O	H	(*R*) -$PhCH_2$	$PhCH_2$	(*R*) -CONHMe
213	O	H	(*R*) -$PhCH_2$	$PhCH_2$	(*S*) -CONHMe
214	O	H	(*S*) -$CH_2CH(Me)_2$	$PhCH_2$	(*R*) -CONHMe
215	O	H	(*S*) -$CH_2CH(Me)_2$	$PhCH_2$	(*S*) -CONHMe
216	O	H	(*R*) -$CH_2CH(Me)_2$	$PhCH_2$	(*R*) -CONHMe
217	O	H	(*R*) -$CH_2CH(Me)_2$	$PhCH_2$	(*S*) -CONHMe
218	H	H	H	Fmoc	(*R*) -CO_2H
219	H	H	H	Fmoc	(*R*) -CO_2Me
220	H	H	H	Fmoc	(*S*) -CO_2H
221	H	H	H	Fmoc	(*S*) -CO_2Me
222	H	H	(*S*)-Me	Fmoc	(*R*) -CO_2H
223	H	H	(*S*)-Me	Fmoc	(*R*) -CO_2Me
224	H	H	(*S*)-Me	$PhCH_2$	(*R*) -CO_2Me
225	H	H	(*R*)-Me	Fmoc	(*R*) -CO_2H
226	H	H	(*R*)-Me	Fmoc	(*R*) -CO_2Me
227	H	H	(*R*)-Me	$PhCH_2$	(*R*) -CO_2Me
228	H	H	(*S*)-Me	Fmoc	(*S*) -CO_2H
229	H	H	(*S*)-Me	Fmoc	(*S*) -CO_2Me
230	H	H	(*S*)-Me	$PhCH_2$	(*S*) -CO_2Me
231	H	H	(*R*)-Me	Fmoc	(*S*) -CO_2H

232	H	H	(*R*)-Me	Fmoc	(*S*) -CO_2Me
233	H	H	(*R*)-Me	$PhCH_2$	(*S*) -CO_2Me
234	H	H	(*S*)- $PhCH_2$	Fmoc	(*R*) -CO_2H
235	H	H	(*S*)- $PhCH_2$	Fmoc	(*R*) -CO_2Me
236	H	H	(*S*)- $PhCH_2$	$PhCH_2$	(*R*) -CO_2Me
237	H	H	(*R*)- $PhCH_2$	Fmoc	(*R*) -CO_2H
238	H	H	(*R*)- $PhCH_2$	Fmoc	(*R*) -CO_2Me
239	H	H	(*R*)- $PhCH_2$	$PhCH_2$	(*R*) -CO_2Me
240	H	H	(*S*)- $PhCH_2$	Fmoc	(*S*) -CO_2H
241	H	H	(*S*)- $PhCH_2$	Fmoc	(*S*) -CO_2Me
242	H	H	(*S*)- $PhCH_2$	$PhCH_2$	(*S*) -CO_2Me
243	H	H	(*R*)- $PhCH_2$	Fmoc	(*S*) -CO_2H
244	H	H	(*R*)- $PhCH_2$	Fmoc	(*S*) -CO_2Me
245	H	H	(*R*)- $PhCH_2$	$PhCH_2$	(*S*) -CO_2Me
246	H	H	(*R*)- CH_2OH	Fmoc	(*S*) -CO_2Me
247	H	H	(*R*)- CH_2OH	$PhCH_2$	(*S*) -CO_2Me
248	H	H	(*R*)- CH_2OBn	Fmoc	(*S*) -CO_2Me
249	H	H	(*R*)- CH_2OBn	$PhCH_2$	(*S*) -CO_2Me
250	H	H	(*R*)- CH_2OH	Fmoc	(*R*) -CO_2Me
251	H	H	(*R*)- CH_2OH	$PhCH_2$	(*R*) -CO_2Me
252	H	H	(*R*)- CH_2OBn	Fmoc	(*R*) -CO_2Me
253	H	H	(*R*)- CH_2OBn	$PhCH_2$	(*R*) -CO_2Me
254	H	H	(*S*)- CH_2OH	Fmoc	(*S*) -CO_2Me
255	H	H	(*S*)- CH_2OH	$PhCH_2$	(*S*) -CO_2Me
256	H	H	(*S*)- CH_2OBn	Fmoc	(*S*) -CO_2Me
257	H	H	(*S*)- CH_2OBn	$PhCH_2$	(*S*) -CO_2Me
258	H	H	(*S*)- CH_2OH	Fmoc	(*R*) -CO_2Me
259	H	H	(*S*)- CH_2OH	$PhCH_2$	(*R*) -CO_2Me
260	H	H	(*S*)- CH_2OBn	Fmoc	(*R*) -CO_2Me
261	H	H	(*S*)- CH_2OBn	$PhCH_2$	(*R*) -CO_2Me
262	H	H	(*S*)-$CH_2CH(Me)_2$	Bn	(*R*) -CO_2Me

263	H	H	(*R*)-$CH_2CH(Me)_2$	Bn	(*R*) -CO_2Me
264	H	H	(*S*)-$CH_2CH(Me)_2$	Bn	(*S*) -CO_2Me
265	H	H	(*R*)-$CH_2CH(Me)_2$	Bn	(*S*) -CO_2Me
266	H	H	(*S*)-$CH_2CH(Me)_2$	Fmoc	(*R*) -CO_2Me
267	H	H	(*R*)-$CH_2CH(Me)_2$	Fmoc	(*R*) -CO_2Me
268	H	H	(*S*)-$CH_2CH(Me)_2$	Fmoc	(*S*) -CO_2Me
269	H	H	(*R*)-$CH_2CH(Me)_2$	Fmoc	(*S*) -CO_2Me
270	H	H	(*S*)-Me	H	(*R*) -CH_2OH
271	H	H	(*S*)-Me	Bn	(*R*) -CH_2OH
272	H	H	(*S*)-Me	Fmoc	(*R*) -CH_2OH
273	H	H	(*R*)-Me	H	(*R*) -CH_2OH
274	H	H	(*R*)-Me	Bn	(*R*) -CH_2OH
275	H	H	(*R*)-Me	Fmoc	(*R*) -CH_2OH
276	H	H	(*S*)-Me	H	(*S*) -CH_2OH
277	H	H	(*S*)-Me	Bn	(*S*) -CH_2OH
278	H	H	(*S*)-Me	Fmoc	(*S*) -CH_2OH
279	H	H	(*R*)-Me	H	(*S*) -CH_2OH
280	H	H	(*R*)-Me	Bn	(*S*) -CH_2OH
281	H	H	(*R*)-Me	Fmoc	(*S*) -CH_2OH
282	H	H	(*S*)-$CH_2CH(Me)_2$	H	(*R*) -CH_2OH
283	H	H	(*S*)-$CH_2CH(Me)_2$	Bn	(*R*) -CH_2OH
284	H	H	(*S*)-$CH_2CH(Me)_2$	Fmoc	(*R*) -CH_2OH
285	H	H	(*R*)-$CH_2CH(Me)_2$	H	(*R*) -CH_2OH
286	H	H	(*R*)-$CH_2CH(Me)_2$	Bn	(*R*) -CH_2OH
287	H	H	(*R*)-$CH_2CH(Me)_2$	Fmoc	(*R*) -CH_2OH
288	H	H	(*S*)-$CH_2CH(Me)_2$	H	(*S*) -CH_2OH
289	H	H	(*S*)-$CH_2CH(Me)_2$	Bn	(*S*) -CH_2OH
290	H	H	(*S*)-$CH_2CH(Me)_2$	Fmoc	(*S*) -CH_2OH
291	H	H	(*R*)-$CH_2CH(Me)_2$	H	(*S*) -CH_2OH
292	H	H	(*R*)-$CH_2CH(Me)_2$	Bn	(*S*) -CH_2OH
293	H	H	(*R*)-$CH_2CH(Me)_2$	Fmoc	(*S*) -CH_2OH

294	H	H	(*S*) -$PhCH_2$	H	(*R*) -CH_2OH
295	H	H	(*S*) -$PhCH_2$	Bn	(*R*) -CH_2OH
296	H	H	(*S*) -$PhCH_2$	Fmoc	(*R*) -CH_2OH
297	H	H	(*R*) -$PhCH_2$	H	(*R*) -CH_2OH
298	H	H	(*R*) -$PhCH_2$	Bn	(*R*) -CH_2OH
299	H	H	(*R*) -$PhCH_2$	Fmoc	(*R*) -CH_2OH
300	H	H	(*S*) -$PhCH_2$	H	(*S*) -CH_2OH
301	H	H	(*S*) -$PhCH_2$	Bn	(*S*) -CH_2OH
302	H	H	(*S*) -$PhCH_2$	Fmoc	(*S*) -CH_2OH
303	H	H	(*R*) -$PhCH_2$	H	(*S*) -CH_2OH
304	H	H	(*R*) -$PhCH_2$	Bn	(*S*) -CH_2OH
305	H	H	(*R*) -$PhCH_2$	Fmoc	(*S*) -CH_2OH
306	H	H	(*R*)- CH_2OH	Fmoc	(*S*) -CH_2OH
307	H	H	(*R*)- CH_2OH	$PhCH_2$	(*S*) -CH_2OH
308	H	H	(*R*)- CH_2OBn	Fmoc	(*S*) -CH_2OH
309	H	H	(*R*)- CH_2OBn	$PhCH_2$	(*S*) -CH_2OH
310	H	H	(*R*)- CH_2OH	Fmoc	(*R*) -CH_2OH
311	H	H	(*R*)- CH_2OH	$PhCH_2$	(*R*) -CH_2OH
312	H	H	(*R*)- CH_2OBn	Fmoc	(*R*) -CH_2OH
313	H	H	(*R*)- CH_2OBn	$PhCH_2$	(*R*) -CH_2OH
314	H	H	(*S*)- CH_2OH	Fmoc	(*S*) -CH_2OH
315	H	H	(*S*)- CH_2OH	$PhCH_2$	(*S*) -CH_2OH
316	H	H	(*S*)- CH_2OBn	Fmoc	(*S*) -CH_2OH
317	H	H	(*S*)- CH_2OBn	$PhCH_2$	(*S*) -CH_2OH
318	H	H	(*S*)- CH_2OH	Fmoc	(*R*) -CH_2OH
319	H	H	(*S*)- CH_2OH	$PhCH_2$	(*R*) -CH_2OH
320	H	H	(*S*)- CH_2OBn	Fmoc	(*R*) -CH_2OH
321	H	H	(*S*)- CH_2OBn	$PhCH_2$	(*R*) -CH_2OH

Table 3



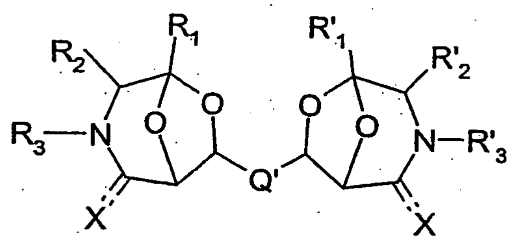
(II)

Compound	R_1	R_2	R_3	R'_1	R'_2	R_6
322	H	H	H	H	H	CO_2Me
323	H	H	H	H	H	CONHMe
324	H	H	$PhCH_2$	H	H	CO_2Me
325	H	H	$PhCH_2$	H	H	CONHMe
326	H	H	Fmoc	H	H	CO_2Me
327	H	H	Fmoc	H	H	CONHMe
328	H	H	Boc	H	H	CO_2Me
329	H	H	Boc	H	H	CONHMe
330	H	$PhCH_2$	H	H	H	CO_2Me
331	H	$PhCH_2$	H	H	H	CONHMe
332	H	$PhCH_2$	$PhCH_2$	H	H	CO_2Me
333	H	$PhCH_2$	$PhCH_2$	H	H	CONHMe
334	H	$PhCH_2$	Fmoc	H	H	CO_2Me
335	H	$PhCH_2$	Fmoc	H	H	CONHMe
336	H	$PhCH_2$	Boc	H	H	CO_2Me
337	H	$PhCH_2$	Boc	H	H	CONHMe
338	H	H	H	H	$PhCH_2$	CO_2Me
339	H	H	H	H	$PhCH_2$	CONHMe
340	H	H	$PhCH_2$	H	$PhCH_2$	CO_2Me
341	H	H	$PhCH_2$	H	$PhCH_2$	CONHMe
342	H	H	Fmoc	H	$PhCH_2$	CO_2Me
343	H	H	Fmoc	H	$PhCH_2$	CONHMe
344	H	H	Boc	H	$PhCH_2$	CO_2Me
345	H	H	Boc	H	$PhCH_2$	CONHMe

346	H	$PhCH_2$	H	H	$PhCH_2$	CO_2Me
347	H	$PhCH_2$	H	H	$PhCH_2$	CONHMe
348	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	CO_2Me
349	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	CONHMe
350	H	$PhCH_2$	Fmoc	H	$PhCH_2$	CO_2Me
351	H	$PhCH_2$	Fmoc	H	$PhCH_2$	CONHMe
352	H	$PhCH_2$	Boc	H	$PhCH_2$	CO_2Me
353	H	$PhCH_2$	Boc	H	$PhCH_2$	CONHMe
354	Ph	H	H	H	H	CO_2Me
355	Ph	H	H	H	H	CONHMe
356	Ph	H	$PhCH_2$	H	H	CO_2Me
357	Ph	H	$PhCH_2$	H	H	CONHMe
358	Ph	H	Fmoc	H	H	CO_2Me
359	Ph	H	Fmoc	H	H	CONHMe
360	Ph	H	Boc	H	H	CO_2Me
361	Ph	H	Boc	H	H	CONHMe
362	H	H	H	Ph	H	CO_2Me
363	H	H	H	Ph	H	CONHMe
364	H	H	$PhCH_2$	Ph	H	CO_2Me
365	H	H	$PhCH_2$	Ph	H	CONHMe
366	H	H	Fmoc	Ph	H	CO_2Me
367	H	H	Fmoc	Ph	H	CONHMe
368	H	H	Boc	Ph	H	CO_2Me
369	H	H	Boc	Ph	H	CONHMe
370	Ph	H	H	Ph	H	CO_2Me
371	Ph	H	H	Ph	H	CONHMe
372	Ph	H	$PhCH_2$	Ph	H	CO_2Me
373	Ph	H	$PhCH_2$	Ph	H	CONHMe
374	Ph	H	Fmoc	Ph	H	CO_2Me
375	Ph	H	Fmoc	Ph	H	CONHMe
376	Ph	H	Boc	Ph	H	CO_2Me

377	Ph	H	Boc	Ph	H	CONHMe
378	H	H	H	H	CH_2OH	CO_2Me
379	H	H	H	H	CH_2OH	CONHMe
380	H	H	$PhCH_2$	H	CH_2OH	CO_2Me
381	H	H	$PhCH_2$	H	CH_2OH	CONHMe
382	H	H	Fmoc	H	CH_2OH	CO_2Me
383	H	H	Fmoc	H	CH_2OH	CONHMe
384	H	H	Boc	H	CH_2OH	CO_2Me
385	H	H	Boc	H	CH_2OH	CONHMe
386	H	$PhCH_2$	H	H	CH_2OH	CO_2Me
387	H	$PhCH_2$	H	H	CH_2OH	CONHMe
388	H	$PhCH_2$	$PhCH_2$	H	CH_2OH	CO_2Me
389	H	$PhCH_2$	$PhCH_2$	H	CH_2OH	CONHMe
390	H	$PhCH_2$	Fmoc	H	CH_2OH	CO_2Me
391	H	$PhCH_2$	Fmoc	H	CH_2OH	CONHMe
392	H	$PhCH_2$	Boc	H	CH_2OH	CO_2Me
393	H	$PhCH_2$	Boc	H	CH_2OH	CONHMe
394	Ph	H	H	H	CH_2OH	CO_2Me
395	Ph	H	H	H	CH_2OH	CONHMe
396	Ph	H	$PhCH_2$	H	CH_2OH	CO_2Me
397	Ph	H	$PhCH_2$	H	CH_2OH	CONHMe
398	Ph	H	Fmoc	H	CH_2OH	CO_2Me
399	Ph	H	Fmoc	H	CH_2OH	CONHMe
400	Ph	H	Boc	H	CH_2OH	CO_2Me
401	Ph	H	Boc	H	CH_2OH	CONHMe

Table 4



(III)

Compound	R_1	R_2	R_3	R'_1	R'_2	R_3	X	Q'
402	H	H	H	H	H	H	O	$CO\text{-}NH(CH_2)_2NH\text{-}CO$
403	H	H	H	H	H	H	O	$CO\text{-}NH(CH_2)_4NH\text{-}CO$
404	H	H	H	H	H	H	O	$CO\text{-}NH(CH_2)_6NH\text{-}CO$
405	H	H	H	H	H	H	O	$CO\text{-}N(C_2H_4)N\text{-}CO$
406	H	H	$PhCH_2$	H	H	$PhCH_2$	O	$CO\text{-}NH(CH_2)_2NH\text{-}CO$
407	H	H	$PhCH_2$	H	H	$PhCH_2$	O	$CO\text{-}NH(CH_2)_4NH\text{-}CO$
408	H	H	$PhCH_2$	H	H	$PhCH_2$	O	$CO\text{-}NH(CH_2)_6NH\text{-}CO$
409	H	H	$PhCH_2$	H	H	$PhCH_2$	O	$CO\text{-}N(C_2H_4)N\text{-}CO$
410	H	H	$PhCH_2$	H	H	$PhCH_2$	H	$CO\text{-}NH(CH_2)_2NH\text{-}CO$
411	H	H	$PhCH_2$	H	H	$PhCH_2$	H	$CO\text{-}NH(CH_2)_4NH\text{-}CO$
412	H	H	$PhCH_2$	H	H	$PhCH_2$	H	$CO\text{-}NH(CH_2)_6NH\text{-}CO$
413	H	H	$PhCH_2$	H	H	$PhCH_2$	H	$CO\text{-}N(C_2H_4)N\text{-}CO$
414	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	O	$CO\text{-}NH(CH_2)_2NH\text{-}CO$
415	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	O	$CO\text{-}NH(CH_2)_4NH\text{-}CO$
416	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	O	$CO\text{-}NH(CH_2)_6NH\text{-}CO$
417	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	O	$CO\text{-}N(C_2H_4)N\text{-}CO$
418	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	H	$CO\text{-}NH(CH_2)_2NH\text{-}CO$
419	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	H	$CO\text{-}NH(CH_2)_4NH\text{-}CO$
420	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	H	$CO\text{-}NH(CH_2)_6NH\text{-}CO$
421	H	$PhCH_2$	$PhCH_2$	H	$PhCH_2$	$PhCH_2$	H	$CO\text{-}N(C_2H_4)N\text{-}CO$
422	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	O	$CO\text{-}NH(CH_2)_2NH\text{-}CO$
423	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	O	$CO\text{-}NH(CH_2)_4NH\text{-}CO$
424	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	O	$CO\text{-}NH(CH_2)_6NH\text{-}CO$
425	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	O	$CO\text{-}N(C_2H_4)N\text{-}CO$
426	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	$CO\text{-}NH(CH_2)_2NH\text{-}CO$

427	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	CO-NH$(CH_2)_4$NH-CO
428	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	CO-NH$(CH_2)_6$NH-CO
429	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	CO-N(C_2H_4)N-CO
430	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	CO-NH$(CH_2)_2$NH-CO
431	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	CO-NH$(CH_2)_4$NH-CO
432	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	CO-NH$(CH_2)_6$NH-CO
433	Ph	H	$PhCH_2$	Ph	H	$PhCH_2$	H	CO-N(C_2H_4)N-CO
434	Ph	H	Ph	Ph	H	Ph	O	CO-NH$(CH_2)_2$NH-CO
435	Ph	H	Ph	Ph	H	Ph	O	CO-NH$(CH_2)_4$NH-CO
436	Ph	H	Ph	Ph	H	Ph	O	CO-NH$(CH_2)_6$NH-CO
437	Ph	H	Ph	Ph	H	Ph	O	CO-N(C_2H_4)N-CO
438	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	O	CO-NH$(CH_2)_2$NH-CO
439	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	O	CO-NH$(CH_2)_3$NH-CO
440	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	O	CO-NH$(CH_2)_4$NH-CO
441	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	O	CO-NH$(CH_2)_5$NH-CO
442	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	O	CO-NH$(CH_2)_6$NH-CO
443	NO_2-Ph	H	Ph	NH_2-Ph	H	Ph	O	CO-N(C_2H_4)N-CO
444	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	O	CO-NH$(CH_2)_2$NH-CO
445	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	O	CO-NH$(CH_2)_3$NH-CO
446	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	O	CO-NH$(CH_2)_4$NH-CO
447	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	O	CO-NH$(CH_2)_5$NH-CO
448	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	O	CO-NH$(CH_2)_6$NH-CO
449	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	O	CO-N(C_2H_4)N-CO
450	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	H	CO-NH$(CH_2)_2$NH-CO
451	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	H	CO-NH$(CH_2)_3$NH-CO
452	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	H	CO-NH$(CH_2)_4$NH-CO
453	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	H	CO-NH$(CH_2)_5$NH-CO
454	NO_2-Ph	H	Ph	NO_2-Ph	H	Ph	H	CO-NH$(CH_2)_6$NH-CO
455	NO_2-Ph	H	Ph	NH_2-Ph	H	Ph	H	CO-N(C_2H_4)N-CO
456	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	H	CO-NH$(CH_2)_2$NH-CO
457	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	H	CO-NH$(CH_2)_3$NH-CO

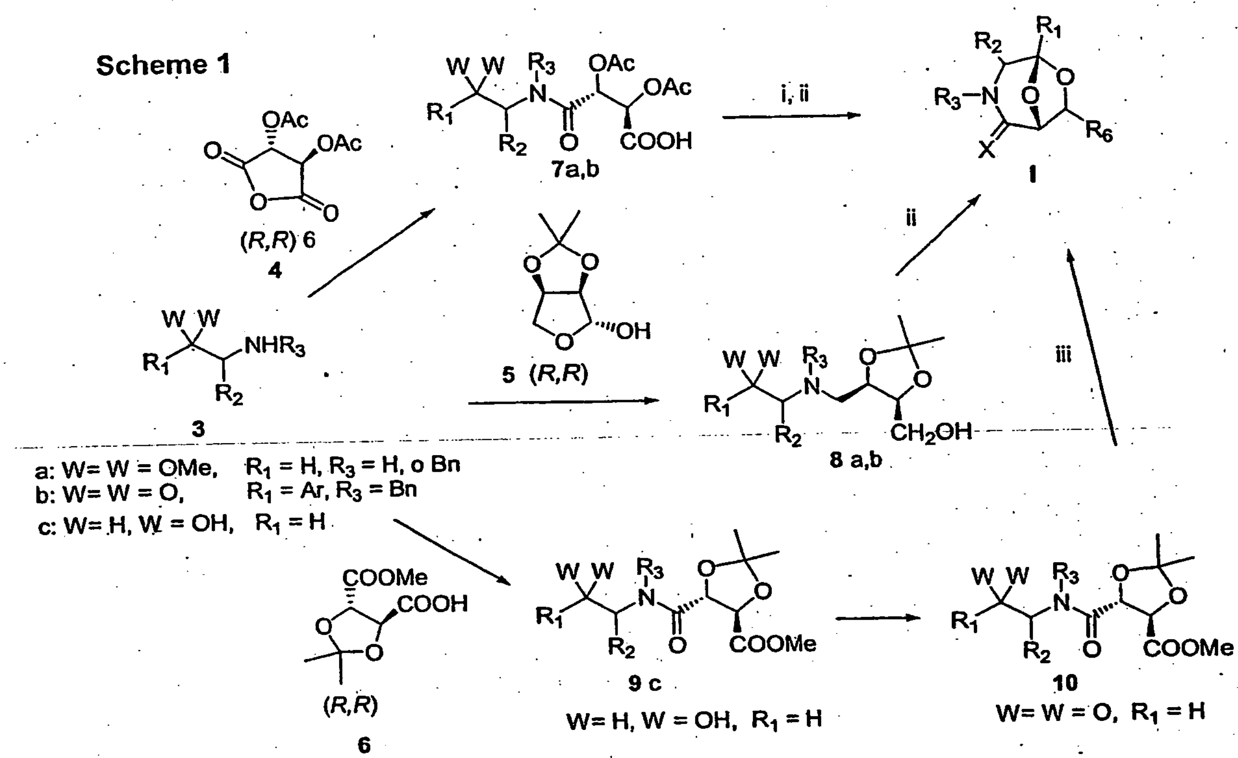
458	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	H	CO-NH(CH_2)$_4$NH-CO
459	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	H	CO-NH(CH_2)$_5$NH-CO
460	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	H	CO-NH(CH_2)$_6$NH-CO
461	NH_2-Ph	H	Ph	NH_2-Ph	H	Ph	H	CO-N(C_2H_4)N-CO

In particular, as far as the dimers of formula (II) and (III) are concerned, all the possible combinations of the stereoisomers are possible, although not exactly specified in the above Table 3 and 4.

Furthermore, the present invention refers to the derivatives of 3-aza-bicyclo[3.2.1]octanes and their dimers that were prepared by the Applicants and described here for the first time, i.e. the 3-aza-bicyclo[3.2.1]octane derivatives (I) and their dimers of general formula (II) and (III) defined as above with exclusion of the following compounds: 1,2,5,7,8,9,10,12,13,17,19,20,21,32,34,35,36,38,40,44, 58,60,64,65,66,70,75,76,77,78,7983,87,91,95,99,101,103,138,145,152,154,163,1 64,168,172,174,176,178,184,186,192,322,324.

The compounds above cited are indeed already described in *J. Org. Chem.* 1999, *64*, 7347, *Organic Letters*, **2000**, *2*, 3987-3990, *Bioorganic & Med Chem* **2001**, *9*, 1625,-1632, *Eur. J. Org. Chem.* **2002**, 873-880, and in the European Application Patent No. 00104135.9-2117 and in the International Application No. WO 01/64686; in such documents the preparation methods of the compounds are also described.

The novel derivatives of 3-aza-bicyclo[3.2.1]octanes of general formula (I) and their dimers of general formula (II) and (III) may be prepared with the following process. The new compounds of general formula (I) and their correspondent dimers of formula (II) and (III), described for the first time in the present application may be prepared according the procedure described as following and represented in the following Scheme 1:

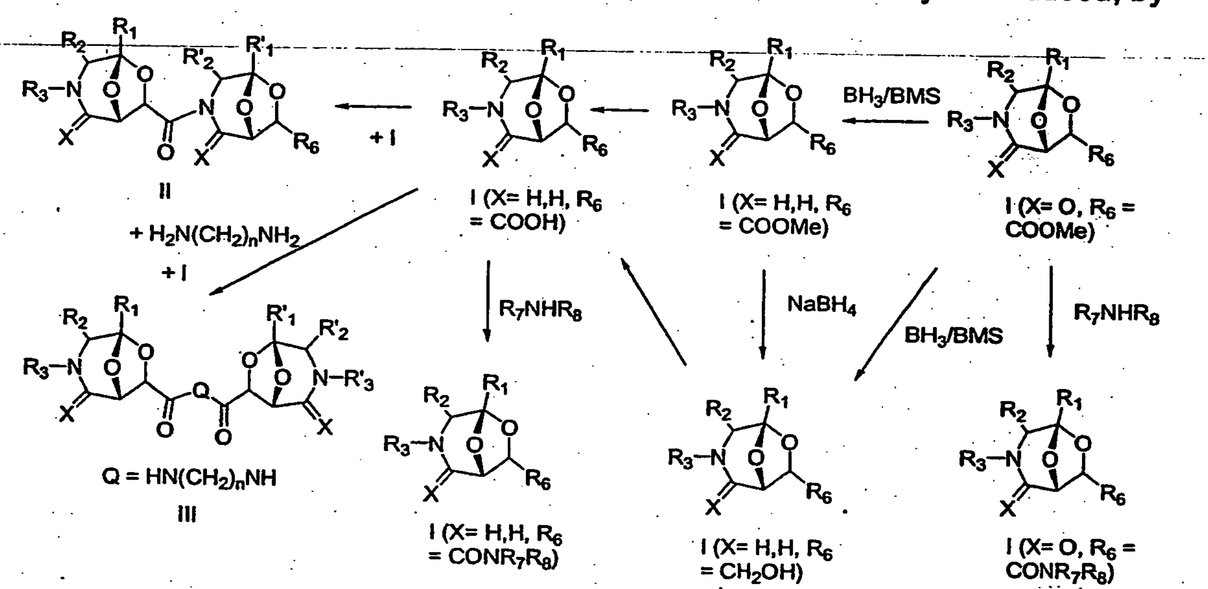


Protected alpha amino aldehydes (**3a**) or alpha amino ketones (**3b**) or alpha amino alcohols (**3c**) were reacted with – activated derivatives of tartaric acid as for example diacetyloxytartaric anydride **4** (*R,R* o *S,S*), - or with acid tartaric derivatives as for example the protected mono-methylester **6** (*R,R* or *S,S*), in the presence of coupling and activating agents - or by reductive amination with protected derivatives of erithrolactole **5** (*R, R* prepared from D-arabinose or S,S prepared from L-arabinose). The correspondent amides **7** e **9** (in the scheme 1 are shown only the *R, R* enantiomers, but the enantiomers S,S were prepared analogously) or amine **8** (in the scheme 1 are shown only the *R,S* enantiomers, but the *S,R* enantiomers were prepared analogously). In the case of amide alcohol **9** the correspondent aldehyde or ketone **10** are obtained by oxidation. When R_3 is H in the amine **8**, a Fmoc protection can be made. The further cyclisation of compounds **7**, **8** e **10** (Scheme 1) occurs by treatment with $SOCl_2$ and MeOH (reaction condition i) followed by treatment with sulfuric acid adsorbed on SiO_2 in refluxing toluene (reaction conditions ii) or by treatment with trifluoro acetic acid (TFA) pure or in methylene chloride (reaction conditions iii). Thus, starting from amides **7** and **10**, the compounds **I** wherein X = O and R_6 = -COOMe in

configuration exo were prepared. In the case of amine 8 compounds I, wherein X = H, H and the group R_6 = $-CH_2OH$ in endo configuration were prepared. The configuration *R,R or S,S* of stereocenters at C-1 bridgehead and at C-7 (bearing the carboxylic or hydroxymethyl group) is depending from that of tartaric acid or from starting erithrolactole. The compounds I may be modified according to Scheme 2.

SCHEME 2

The compounds of formula (I) (amide type), wherein X = O may be reduced, by



using the complex BH_3 dimethyl sulfide, either to correspondent amino esters I (X = H, H, R_6 = COOMe), or to correspondent amino alcohol I (X=H, H e R_6 = CH_2OH). Such compounds may be deprotected to nitrogen atom. The hydrolysis of amino ester I (X = H, H, R_6 = COOMe) may be done either in acid or basic conditions, giving to the correspondent amino acid I (X = H, H, R_6 = COOH). The amino acid is also obtained by Jones oxidation or by using PDC in DMF, from amino alcohol I (X=H, H e R_6 = CH_2OH), also after the change of the benzyl group to Boc or Fmoc. By activation of the carboxylic group an amide bond with an amine NHR_7R_8 or an amino acid is formed. Otherwise, the activated carboxylic group of the amino acid I, is reacted with another unit of I having the deprotected nitrogen, to give the dimers of general formula (II) present in Table 3.

Otherwise, two units of a compound of formula (I) in each form, is reacted with a spacer Q, to give the dimers of general formula (III). The example shown in the scheme 2 includes but is not limited to the reaction of a diamine (Q) with two units of an activated carboxylic acid to give dimers of formula (III) reported in Table 4 .

The present 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I) and their dimers of general formula (II) and (III), in free form or in form of pharmaceutically acceptable salts, may be used for preparation of pharmaceutical compositions following usual methods of pharmaceutical preparation.

Such pharmaceutical compositions may be formulated in conventional way, and may include one or more eccipients and/or diluent pharmaceutically acceptable.

Administration of such formulations is feasible through any conventional route, such as parenteral, in the form of solution or suspension, oral, ocular, nasal, topical, etc.

The formulation of the 3-aza-bicyclo[3.2.1]octane derivatives of formula (I) and of their dimers of formula (II) and (III) according to the invention include tablets, capsules, pills, pellets, solutions, dispersions, suspensions, liposomal formulations, microspheres, nanospheres, creams and ointments, emulsions and aerosols, that can also be prepared in a way that allows a controlled or retarded release of the active compound.

Such pharmaceutical compositions may comprise at least one among the present compounds of formula (I), (II) and (III), or mixtures thereof, as active principle, possibly even in combination with other active principle or co-adjuvant, selected according to the pathologic conditions.

The pharmaceutical compositions comprising the compounds of the invention are suitable for pharmaceutical treatment of pathologic conditions related to the activity of neurotrophins.

The present derivatives of 3-aza-bicyclo[3.2.1] octane derivatives of general formula (I) and their dimers of general formula (II) showed neurotrophin agonist activity, especially of NGF, as they have the property of interacting with the NGF receptor complex at defined affinity levels. The agonist compounds have the property of inducing the biological signal of neurotrophins. The neurotrophin

agonist compounds are suitable for, e.g., preparation of pharmaceutical compositions useful in the treatment of:

i) neurodegenerative, inflammatory, toxic, traumatic, or vascular disorders of the central, peripheral, or autonomic nervous system (such as Alzheimer Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Huntington disease, multiple sclerosis, epilepsy, Down syndrome, nervous deafness, Ménière's disease), neural damages secondary to hypoxia, ischaemia, burns, chemotherapy, toxic compounds of various origin (including alcohol), infections (such as polio or HIV virus), trauma (including surgical trauma) originating axotomy of motoneurons, sensorial, motor, or sensorimotor neuropathies, or autonomic dysfunctions secondary to diverse pathologies (such as diabetes, renal insufficiency, or other systemic diseases), genetic disorders (such as Charcot-Marie-Tooth disease, Refsum disease, abetalipoprotenemia, Tangier disease, Krabbe disease, metachromatic leukodystrophy, Fabry disease, Dejerine-Sottas disease), nervous pathologies of diverse origin (such as diffuse athrophy of cerebral cortex, Lewy body dementia, Pick's disease, mesolimbocortical dementia, neuronal ceroid lipofuscinosis, thalamic degeneration, cortico-striatal-spinal degeneration, cortico-basal ganglionic degeneration, cerebro-cerebellar degeneration, familial dementia with spastic paraparesis, polyglucosan bodies disease, Shy-Drager synfrome, olivopontocerebellar atrophy, progressive supranuclear palsy, deforming muscular dystony, Hallervorden-Spatz disease, Meige's syndrome, familial shivering, Gilles de la Tourette syndrome, chorea-acanthocytosis syndrome, Friedreich's ataxia, Holmes' corticocerebellar familial atrophy, Gerstmann-Straussler-Scheinker disease, progressive spinal muscular atrophy, spastic paraplegia, peroneal muscular atrophy, hypertrophic interstitial polyneuropathy, polyneuritic ataxic heredopathy), some ocular pathologies (such as optic nerve neuropathies, retinal degeneration, ophtalmoplegy, glaucoma), corneal diseases of diverse origin (such as neurotrophic ulcers, post-traumatic or post-infective corneal disorders), pathologies from reduced motility of the gastro-intestinal tract or from urinary bladder atony (such as interstitial cystitis or diabetic cystitis), endocrine neoplastic pathologies (such as prolactinoma), clinical conditions in which stimulation of learning processes is advantageous (in particular, in dementias and in post-

traumatic conditions), besides all pathological conditions originating from apoptotic processes of neural cells;

ii) acquired immunodeficiency diseases due to reduced or absent bioavailability of NGF (such immunodificiency of ageing);

iii) conditions in which stimulation of neoangiogenesis may be advantageous (such as myocardial infarction, stroke, cerebral aneurysms, gastro-duodenal ulcers, wound healing, peripheral vasculopathies);

iv) certain ocular pathologies (such as corneal pathologies of diverse origin and glaucoma).

The present 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I), and their dimers of general formula (II) and (III) above reported, are also suitable for the preparation of culture and storage media useful for conservation of explanted corneas destined to transplantation.

Moreover, when labelled with suitable reagents (contrast agents, radioisotopes, fluorescent agents, etc.), and possibly processed with any other procedure useful for medical imaging purposes, the present 3-aza-bicyclo[3.2.1]octane derivatives of general formula (I), and their dimers of general formula (II) and (III), may be used for the imaging analysis of tissues and organs containing neurotrophine receptors, either *in vitro* or *in vivo*. In particular such labelled compounds may be used either for monitoring the use and efficacy of drugs or for the diagnosis of mammal diseases in which the neurothrophine receptors are involved.

In general, the present compounds having neurotrophin agonistic activity, in particular NGF agonistic activity, were proven adequate to substitute for neurotrophin and NGF biologic activity.

Furthermore, the present neurotrophin agonistic compounds can be used to promote *in vivo*, *in vitro*, or *ex vivo* growth and/or survival of neural cells, including, but not limited to: dopaminergic, cholinergic, sensorial neurons, striatal cells, cortical cells, cells of the corpus striatum, hippocampus, cerebellum, olfactory bulbs, peri-aqueductal cells, cells of the raphe nuclei, of the locus coeruleus, of the dorsal root ganglia, sympathetic neurons, lower motoneurons, nervous stem cells, or cells anyhow deriving from the neural plaque.

The following examples are reported to give a non-limiting illustration of the

present invention.

EXAMPLE 1

Preparation of methyl 3-benzyl-2-oxo-(1*S*,5*S*,7*R*)-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (compound of formula (I) where X = O, R_1 = H, R_2 = Bn, R_6 = (*R*)-COOMe) (Compound 1)

A solution of *R,R* tartaric anhydride **4** (4 g) (prepared as reported by Lucas H.J., Baumgarten W., *J.Am.Chem.Soc.*, 1941, *63*, 1654) in anhydrous dichloromethane (23 ml) and **3a** (where X = X = OMe, R_1 = H, R_2 = H, R_3 = Bn,) (3 g) prepared as reported (Kermak, W. O.; Perkin, W. H.; Robinson, R. *J. Chem. Soc.*, *Trans*, 1922, *121*, 1872) were reacted at r. t. for 15 h. After evaporation of the solvent **7a** (7 g), is obtained as an oil. To the crude product **7a** in CH_3OH (40 ml), thionyl chloride is added dropwise (0.8 ml) at 0 °C and then the mixture heated at 60 °C for 15 h. After evaporation of solvent, the crude product dissolved in toluene (8 ml) is quickly added to a refluxed suspension of (1.6 g) H_2SO_4/SiO_2 (H_2SO_4 30% by weight) in toluene (12.5 ml). After 15 min, one third of the solvent is distilled off and the remaining hot mixture is filtered on a short pad of $NaHCO_3$. After evaporation of the solvent, the crude product was purified by chromatography giving the pure compound of the title (2.8 g).

^{1}H NMR ($CDCl_3$) δ 7.32-7.16 (m, 5H), 5.84 (d, *J*=2.0 Hz, 1H), 4.96 (s, 1H), 4.74 (s, 1H), 4.52 (s, 2H), 3.77 (s, 3H), 3.34 (dd, J_1=12.0 Hz, J_2=2.0 Hz, 2H), 3.08 (*J*=12.0 Hz, 1H). P.f. 82, $[\alpha]^{25}_D$ = - 49 (c 1.0, $CHCl_3$)

EXAMPLE 2

Preparation of methyl (1R,5R,7S)-3-benzyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1] octane-7-*exo*-carboxylate (compound of formula (I) where X = O, R_1 = R_2 = H, R_3 = Bn, R_6 = (*S*)-COOMe) (Compound 191)

Following the same procedure of Example 1, starting from anhydride *S,S* tartaric **4**, the compound of the title is obtained.

^{1}H NMR ($CDCl_3$) δ 7.40-7.10 (m, 5H), 5.85 (d, *J*=2.0 Hz, 1H), 4.97 (s, 1H), 4.74 (s, 1H), 4.52 (s, 2H), 3.79 (s, 3H), 3.34 (dd, J_1=12.0 Hz, J_2=2.0 Hz, 2H), 3.09 (*J*=12.0 Hz, 1H). P.f. 83, $[\alpha]^{25}_D$ = + 48 (c 1.0, $CHCl_3$)

EXAMPLE 3

Preparation of methyl (1S,5S,7R)-3-benzyl-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (compound of formula (I) where X = R_1 = R_2 = H, R_3 = Bn, R_6 = (*R*)-COOMe) (compound 40)

A solution of $BH_3 \cdot Me_2S$ (1 M, 2.5 ml,) was slowly added at 0°C to a solution in anhydrous THF (65 ml) of compound of formula (I) where X = O, R_1 = H, R_2 = H, R_3 = Bn, R_6 = (*R*)-COOMe (compound 1) (2.8 g) prepared as described above in Example 1. The mixture was stirred for 18 h at r. t. and then ethanol (3 ml), NaOH solution (3M, 2 ml) and H_2O (150 ml) were added. After extraction with diethylether, the organic phase was separated and evaporated giving, after chromatography, the pure compound of the title (2 g) as colorless oil.

^1H NMR ($CDCl_3$) δ 7.30-7.23 (m, 5H), 5.62 (s, 1H), 4.78 (s, 1H), 4.60 (s, 1H), 3.74 (s, 3H), 3.55 (pd, 2H), 2.84 (d, J=13 Hz, 1H), 2.76 (d, J=10 Hz, 1H), 2.50 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 2.30 (d, J=11 Hz, 1H). $[\alpha]^{25}_D$ = - 60 (c 1.0, $CHCl_3$).

EXAMPLE 4

Preparation of methyl (1S,5S,7R)-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (compound of formula(I) where X = R_1 = R_2 = R_3 = H, R_6 = (*R*)-COOMe) (Compound 34)

To a suspension of compound of formula (I) where X = R_1 = R_2 = H, R_3 = Bn, R_6 = (*R*)-COOMe) (compound 40) (2 g) prepared as described above in Example 3 and Pd/C 10 % (1.3 g) in methanol (40 ml), is added ammonium formiate (2.4 g). The mixture left at reflux for 1h, was filtered on Celite and washed with CH_3OH. The solution is evaporated to give the compound of the title (1.3 g), as colorless oil. ^1H NMR ($CDCl_3$) δ 5.53 (s, 1H), 4.72 (s, 1H), 4.49 (s, 1H), 3.71 (s, 3H), 3.17 (dd, J_1=13.6 Hz, J_2=1.8 Hz, 1H), 2.83 (m, 2H), 2.68 (d, J=13.6 Hz, 1H), 2.55 (br, 1H). $[\alpha]^{25}_D$ = - 55 (c 0.7, $CHCl_3$).

EXAMPLE 5

Preparation of acid (1S,5S,7R)-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylic (compound of formula (I) where X = R_1 = R_2 = R_3 = H, R_6 = (*R*)-COOH) (Compound 32)

The compound of formula (I) where X = R_1 = R_2 = R_3 = H, R_6 = (*R*)-COOMe (Compound 34) prepared as described in Example 4 (0.5 g) was dissolved in a

solution of HCl (4N, 12 ml). After 18 h at r. t., the solution was evaporated obtaining the title compound as HCl salt (0.5 g).

$[\alpha]^{25}_D$ -38.3 (*c* 1.1, H_2O); ^{1}H NMR (D_2O) δ 5.95 (s, 1H), 5.06 (s, 1H), 5.04 (s, 1H), 3.58 (m, 2H), 3.34 (m, 2H);

EXAMPLE 6

<u>Preparation of methyl (1S,5S,7R)-3-*ter*-butoxycarbonyl-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (compound of formula (I) where X = R_1 = R_2 = H, R_3 = Boc, R_6 = (*R*)-COOMe) (Compound 42)</u>

DIPEA (0.8 ml) and $(BOC)_2O$ (1.1 g) were added to a solution in CH_2Cl_2 anhydrous (9 ml) and ethanol (3 ml) of the compound of formula (I) wherein X = R_1 = R_2 = R_3 = H, R_6 = (*R*)-COOMe (Compound 34) (0.8 g) prepared as described in Example 4. The reaction mixture was left for 18 h at r. t., the solvent was evaporated and the residue was treated with a solution of $NaHSO_3$ (5 %) and extracted with diethylether. After evaporation of the solvent, the crude product was purified by chromatography to give the title compound (0.8 g) as white solid.

^{1}H NMR ($CDCl_3$) δ 5.64 and 5.58 (rotamers) (s, 1H), 4.65 and 4.60 (rotamers) (s, 1H), 4.51 (s, 1H), 3.72 (s, 3H), 4.00-3.60 (m, 2H), 3.20 (m, 1H), 2.92 (m, 1H), 1.43 (s, 9H).

EXAMPLE 7

<u>Preparation of (1S,5S,7R)-3-*ter*-butoxycarbonyl-6,8-dioxa-7-*exo*-hydroxymethyl-3-azabicyclo[3.2.1]octane (compound of formula (I) where X = R_1 = R_2 = H, R_3 = Boc, R_6 = (*R*)-CH_2OH) (Compound 62)</u>

To a solution in MeOH (15 ml) of the compound of formula (I) where X = R_1 = R_2 = H, R_3 = Boc, R_6 = (*R*)-COOMe) (Compound 42) (0.8 g) prepared as described in Example 6, at 0 °C, $NaBH_4$ (0.6 g) was added in small portions. After 10 min at r. t., the mixture was evaporated, and the crude product was purified by chromatography to give the compound of the title (0.5 g) as a colourless oil. $[\alpha]^{25}_D$ −30 (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$) δ 5.50 and 5.44 (rotamers) (s, 1H), 4.32 and 4.27 (rotamers) (s, 1H), 4.18 (m, 1H), 3.88-3.67 (m, 2H), 3.56 (d, *J*=5.5 Hz, 2H), 3.21 (m, 1H), 2.96 (m, 1H), 1.92 (b, 1H), 1.43 (s, 9H).

EXAMPLE 8

<u>Preparation of (1*S*,5*S*,7*R*)-3-(9-Fluorenylmethoxycarbonyl)-7-*endo*-hydroxymethyl-6,8-dioxa-3-aza-bicyclo[3.2.1]octane (compound of formula (I) where X = R_1 = R_2 = H, R_3 = Fmoc, R_6 = (*R*)-CH_2OH) (compound 61)</u>

To a solution of 2,3-*O*-isopropylidene-D-erithrose (*R,R*) **5** (1,8 g) in THF (prepared from D-Arabinose, as reported by Thompson, D.K.; Hubert, C.N.; Wightman, R.H. *Tetrahedron* 1993, *49*, 3827-3840) 2,2-diethoxyethylamine **3a** (where W= W= OEt, R_1 = R_2 = R_3 =H) (1,7 ml) a 0 °C, $NaBH(OAc)_3$ (3.1 g) was added in small portions. After 18h a r. t., the mixture is diluted with a saturated solution of $NaHCO_3$ and extracted with ethyl acetate. The organic phase was evaporated giving an oil, which was chromatographed to give the product **8a** (where W= W= OEt, R_1=R_2=R_3=H) as yellowish oil (1.9 g).

$[\alpha]^{20}_D$ -8.4 (*c* 0.54, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.83 (br, 2 H), 4.59 (t, *J* = 5.5 Hz, 1 H), 4.32 (m, 2 H), 3.75-3.45 (m, 6 H), 3.05-2.83 (m, 2 H), 2.79 (d, *J* = 5.5 Hz, 2 H), 1.44 (s, 3 H), 1.34 (s, 3 H), 1.21 (t, *J* = 7.0 Hz, 6 H).

To a solution of **8a** (where W= W= OEt, R_1=R_2=R_3=H) (1.7 g) in acetone (40 ml) Fmoc-*O*-Su (2.1 g) and an aqueous solution of $Na_2CO_3 \cdot H_2O$ (0.75 g in 40 ml) were added at 0°C. The mixture was left at r.t. for 18 h, and extracted with CH_2Cl_2, then the solvent was evaporated and the residue was chromatographed to give the product **8a** (where W= W= OEt, R_1=R_2=H, R_3 =Fmoc) as yellowish oil (2.2 g).

$[\alpha]^{20}_D$ -34 (*c* 0.38, MeOH); ^1H NMR ($CDCl_3$) δ7.73 (d, J = 7.3 Hz, 2 H), 7.56 (m, 2 H), 7.34 (m, 4 H), 4.63 (m, 2 H), 4.47-4.14 (m, 3 H), 4.19 (t, *J* = 4.9 Hz, 1 H), 3.74-3.02 (m, 10 H), 1.42-1.04 (m, 12 H);

Compound **8a** (where W= W= OEt, R_1=R_2=H, R_3 =Fmoc) (1.9 g) dissolved in trifluoroacetic acid (8 ml) was left aside for 18 h a r. t. After evaporation of TFA, the crude compound, dissolved in MeOH, was filtered on as short pad of $NaHCO_3$, then the solvent was evaporated and the residue was chromatographed to give the title product as a white solid (1 g).

M.p. 41-42 °C; $[\alpha]^{20}_D$ -32 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ7.77 (d, *J* = 7.0 Hz, 2 H), 7.57 (d, *J* = 7.0 Hz, 2 H), 7.38 (m, 4 H), 5.51 (s, 1 H), 4.92-2.95 (m, 12 H).

EXAMPLE 9

Preparation of acid (1*S*,5*S*,7*S*)-3-(9-Fluorenylmethoxycarbonyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octan-7-*endo* carboxylic (compound of formula(I) where X = R_1 = R_2 = H, R_3 = Fmoc, R_6 = (*S*)-COOH) (Compound 39)

To a solution of the compound of formula (I) where X = R_1 = R_2 = H, R_3 = Fmoc, R_6 = (*R*)-CH_2OH (compound 61) (0.9 g) prepared according to the Example 8, in acetone (75 ml) was added the Jones reagent at 0°C, [prepared by slow addition of H_2SO_4 (2.8 ml) to a solution of CrO_3 (1.5 g) in H_2O (20 ml) a 0°C]. The mixture was left for 18 h at r.t and then was added with isopropanol, filtered on Celite and evaporated. The crude product dissolved in EtOAc (45 ml) was extracted with 10% $NaHCO_3$ in water. After separation, the aqueous phase was acidified at pH 1 with HCl and extracted with EtOAc. Evaporation of the organic phase gave a crude product which was chromatographed to give the compound of the title (0.7 g) as a white solid.

M.p. 79-82°C; $[\alpha]^{20}_D$ -53 (*c* 0.5, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 7.75 (m, 2H); 7.53 (d, J = 7.0 Hz, 2H); 7.38 (m, 4H); 5.56 (s, 1H); 4.74-4.45 (m, 4H); 4.23-3.91 (m, 4H); 3.29-3.11 (m, 2H).

EXAMPLE 10

Preparation of (1*R*,5*R*,7*R*)-3-(9-Fluorenylmethoxycarbonyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octan-7-*endo* carboxylic acid (compound of formula (I) where X = R_1 = R_2 = H, R_3 = Fmoc, R_6 = (*R*)-COOH) (compound 218)

A solution of (1*R*,5*R*,7*S*)-3-(9-fluorenylmethoxycarbonyl)-7-*endo*-hydroxymethyl-6,8-dioxa-3-aza-bicyclo[3.2.1]octane (compound of formula (I) where X = R_1 = R_2 = H, R_3 = Fmoc, R_6 = (*S*)-CH_2OH) (1.8 g), prepared from (*S,S*) erythrose 5 (obtained starting from L-arabinose) with the same procedure above described in the Example 8 for its enantiomer, was treated as above described in the Example 9 for its enantiomer, to give 1.4 g of the title compound as white solid.

M.p. 71-81 °C; $[\alpha]^{20}_D$ +52.9 (*c* 0.50, $CHCl_3$).

EXAMPLE 11

Preparation of methyl 3-benzyl-5-phenyl-2-oxo-(1S,5S,7R)-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (compound of formula (I) where X = O, R_1 = Ph, R_2 = H, R_3 = Bn, R_6 = (*R*)-COOMe) (Compound 27)

To a solution of **3b** (2.4 g) (where X = O, R_1 = Ph, R_2 = H, R_3 = Bn,) (prepared according the procedure reported by R Simonoff and W.H Hartung, *J. Am. Pharm. Assoc., 35*, 306, 1946) in dry CH_2Cl_2 (20 ml), (*R,R*) **6** acid tartaric derivative (2,49 g, 5,33 mmol) and DIPEA (5,4 ml) were added. The mixture was stirred at r. t. for 2 h, the solvent was evaporated to give an oil which was extracted in ethyl acetate. The solution was washed with solution of 5% $KHSO_4$,and 5% $NaHCO_3$ in water. After evaporation of the solvent the residue was purified by chromatography to give **8b** (where X = O, R_1 = Ph, R_2 = H, R_3 = Bn,) (3.2 g) as colourless oil.

^{1}H NMR δ 7.90-7.85 (m, 2 H), 7.61-7.22 (m, 8 H), 5.39 (d, *J* = 5.1 Hz, 1 H), 5.11 (d, *J* = 5.1 Hz, 1 H), 4.88-4.10 (m, 4 H), 3.80 (s, 3 H), 1.49 (s, 3 H), 1.31 (s, 3 H).

A solution of **8b** (3.2 g) (where X = O, R_1 = Ph, R_2 = H, R_3 = Bn,) in toluene (80 ml) was quickly added to a suspension of H_2SO_4/SiO_2 (30% w/w, 1.4 g) in toluene at reflux (120 ml). After 15 min one third of the solvent was distilled off and the hot remaining mixture was filtered on a short pad of $NaHCO_3$. After evaporation of the solvent the residue was purified by chromatography to give 2.4 g of the title compound as colorless solid.

M.p. 113-114 °C. $[\alpha]^{25}_D$ -64.0 (*c* 1, $CDCl_3$). ^{1}H NMR δ 7.62-7.59 (m, 2 H), 7.41-7.24 (m, 8 H), 5.16 (s, 1 H), 4.92 (s, 1 H), 4.61 (m, 2 H), 3.74 (s, 3 H), 3.46 (m, 2 H).

EXAMPLE 12

Preparation of methyl 3-benzyl-5-phenyl-(1S,5S,7R)-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (compound of formula (I) where X = R_2 = H, R_1 = Ph, R_3 = Bn, R_6 = (*R*)-COOMe) (Compound 120)

To a solution in dry THF (25 ml) of the compound of formula (I) where X = O, R_1 = Ph, R_2 = H, R_3 = Bn, R_6 = (R)-COOMe) (compound 27) prepared as described in Example 11 (2,5 mmol), at 0°C, $BH_3{\cdot}Me_2S$ (10 M 0,5 ml, 4.9 mmol) was added dropwise. The mixture was left aside for 16 hr and then EtOH (1 ml), 3 M NaOH (1 ml) and H_2O (20 ml) were added. After extraction with diethylether, and evaporation of the solvent the residue was purified by chromatography to give 1 g of the compound of the title as colorless solid.

M.p. 97 °C. $[\alpha]^{25}_D$=13.0 (c 1, $CHCl_3$). ^{1}H NMR δ 7.72-7.58 (m, 2 H), 7.52-7.19 (m, 8 H), 5.00 (s, 1 H), 4.86 (s, 1 H), 3.75 (m 2 H), 3.78 (s, 3 H), 3.62 (m, 2 H), 3.16 (d, *J*= 11.2, 4 H), 2.93 (d, *J*= 11.6, 2 H), 2.63 (d, *J*= 11.0, 2 H).

EXAMPLE 13

Preparation of methyl (1*S*,4*S*,7*R*)-3,4-Dibenzyl-2-oxo-6,8-dioxa-3-azabicyclo[3,2,1]octane-7-*exo*-carboxylate (compound of formula (I) where X = O, R_1 = H, R_2 = (*S*)Bn, R_3 = Bn, R_6 = (*R*)-COOMe) (Compound 12)

To a solution of L-phenylalaninol **3c** (where W=H, W=OH, R_1 =H, R_2 = Bn, R_3 = H) (5 g) in MeOH (150 ml) benzaldehyde (3.3 ml) were added. The reaction mixture was stirred at r. t. for 1 h, then 1.2 g of $NaBH_4$, were added in small portions in 2 hr at 0°C. The solvent was evaporated and the residue extracted with 50 ml of HCl at pH=2. The aqueous solution was extracted with Et_2O, treated with Na_2CO_3 until pH=9 and then extracted with $CHCl_3$. The organic phase evaporated gave *N*-benzyl-(L)-phenylalaninol as white solid (7 g) **3c** (where W=H, W=OH, R_1 =H, R_2 = Bn, R_3 = Bn)

^{1}H NMR ($CDCl_3$) δ, ppm: 7.34-7.06 (m, 10 H), 3.73 (s, 2 H), 3.31 (dd, J = 6.2, 12.5 Hz, 1 H), 3.00-2.81 (m, 1H), 2.80-2.66 (m, 2 H). 2.62 (dd, *J* = 6.2, 12.5 Hz,1 H)

To a solution of *N*-benzyl-(L)-phenylalaninol **3c** (2.8 g) in 23 ml of $CHCl_3$ at 0°C , DIPEA (4 ml), HOBt (2.1 ml) and a solution of methyl ester of (2*R*, 3*R*)-2,3-*O*-isopropylidentartaric acid **(6)** (2.4 g) in 23 ml of $CHCl_3$, were added. Then 1.7 g of DIPC were added. After 72 hr at r. t., the solvent was evaporated and the crude product residue was purified by chromatography to give a yellowish solid (2.4 g) **9c** (where W=H, W=OH, R_1 =H, R_2 = Bn, R_3 = Bn).

$[\alpha]_D^{25}$ - 72 (c=0,5 , $CHCl_3$). ^{1}H NMR ($CDCl_3$), δ, ppm: (mixture of rotamers 2:1) major δ 7,40-7,05 (m, 10 H), 5,28 (d, *J* = 6,0 Hz, 1 H), 4,81 (d, *J* = 6,0 Hz, 1 H), 4,75 (d, *J* = 16,4 Hz, 1 H), 4,0 (d, *J* = 16,4 Hz, 1 H), 3,79 (s, 3 H), 3,70 (m, 1 H), 3,60 (m, 1 H), 3,46 (m, 1 H), 3,04 (m, 1 H), 1,52 (s, 3 H), 1,49 (s, 3 H).

The compound **9c** (where W=H, W=OH, R_1 =H, R_2 = Bn, R_3 = Bn) was oxidized to **10** (where W=O, W=O, R_1 =H, R_2 = Bn, R_3 = Bn) by Swern oxidation. 4.5 g of alcohol **(9c)** in 20 ml of CH_2Cl_2 were oxidized as usual by treatment with oxalyl chloride, DMSO and DIPEA. After usual work-up compound **(10)** (5 g) was obtained as yellow solid.

^{1}H NMR ($CDCl_3$) δ ppm: 9,44 (s, 1 H), 7,40-7,00 (m, 10 H), 5,33 (d, *J* = 6,2 Hz, 1 H), 4,92 (d, *J* = 6,2 Hz, 1 H), 4,89 (d, *J* = 18,7 Hz, 1 H), 3,79 (s, 3 H), 3,53 (dd, *J* =

9,8, 4,3 Hz, 1 H), 3,44 (d, J = 18,7 Hz, 1 H), 3,41 (dd, J = 13,9, 4,3 Hz, 1 H), 3,12 (dd, J = 13,9, 9,8 Hz, 1 H), 1,54 (s, 3 H), 1,45 (s, 3 H).

The product was added in toluene (15 ml), to a suspension of 2.5 g SiO_2 and H_2SO_4 in 30 ml of refluxing toluene; After 30 min, After 15 min one third of the solvent was distilled off and the hot remaining mixture was filtered on a short pad of $NaHCO_3$. After evaporation of the solvent the residue was purified by chromatography to give 3.2 g of the title compound.

^{1}H NMR ($CDCl_3$) δ ppm: 7,40-7,15 (m, 8 H), 7,03 (m, 2 H), 5,51 (s, 1 H), 5,33 (d, J = 15,0 Hz, 1 H), 4,97 (s, 1 H), 4,71 (s, 1 H), 4,03 (d, J = 15,0 Hz, 1 H), 3,75 (s, 3 H), 3,32 (dd, J = 10,7, 3,7 Hz, 3 H), 3,15 (dd, J = 13,5, 3,7 Hz, 1 H), 2,75 (dd, J = 13,5, 10,7 Hz, 1 H)

EXAMPLE 14

Preparation of (1*S*,4*S*,7*R*)-3,4-Dibenzyl-6,8-dioxa-7-*exo*-hydroxymethyl 3-azabicyclo[3,2,1]octane (compound of formula (I) where X = R_1 = H, R_2 = (*S*)Bn, R_3 = Bn, R_6 = (*R*)-CH_2OH) (Compound 184)

To a solution in 100 ml of anhydrous THF of the compound of formula (I) where X = O, R_1 = H, R_2 = (*S*)Bn, R_3 = Bn, R_6 = (*R*)-COOMe (compound 12) (4 g), prepared as described in Example 13, a solution $BH_3{\cdot}SMe_2$ (3 ml, 10 M) in THF was added. After 38 hr at r. t. the reaction mixture was treated with dry EtOH (6ml) and 10% of NaOH (6 ml), then diluted with 50 ml of water and extracted with Et_2O. After evaporation of the solvent the residue was purified by chromatography to give 1.7 g of the title compound as yellowish solid. $[\alpha]_D^{25}$ -59 (c = 0,2, $CHCl_3$)

^{1}H NMR ($CDCl_3$) δ, ppm: 7,40-7,00 (m, 10 H), 5,11 (s, 1 H), 4,39 (t, J = 5,1 Hz, 1 H), 4,24 (s, 1H), 3,81 (d, J = 13,6 Hz, 1 H), 3,63 (d, J = 13,6 Hz, 1 H) 3,52 (m, 2 H), 3,00 (m, 1 H) 3,00-2,80 (m, 2 H), 2,94 (d, J = 11,6 Hz, 1 H), 2,45 (dd, J = 11,6, 1,8 Hz, 1 H)

EXAMPLE 15

Preparation of dimer of formula (II) where R_1 = R_1' = H, R_2 = R_3 = R_2' = Bn, R_6 =(*R*)-COOMe (Compound 348)

0.1 ml of DIPEA were added to a solution in 0.3 ml of CH_2Cl_2 of the compound of formula (I) where X = R_1 = H, R_2 = (*S*)-Bn, R_3 = Bn, R_6 =(*R*)-COOH (Compound 188) (0.1 g) obtained by hydrolysis of the corresponding methyl ester (Compound

172) according to the procedure in Example 5. Then, 0.2 g of PyBroP at 0°C and 0.05 g (0.209 mmol) of the compound of formula (I) where $X = R_1 = R_3 = H$, $R_2 =$ (*S*)-Bn, $R_6 =$(*R*)-COOMe (Compound 178) were added. The mixture was stirred overnight, the solvent evaporated and the residue dissolved in 50 ml of AcOEt. After evaporation of the solvent the residue was purified by chromatography to give 0.07 g of the title compound as white solid.

EXAMPLE 16

<u>Preparation of dimer of formula (III) where $X = O$, $R_1 = R_1' =$ p-NO_2Ph, $R_2 = R_2' =$ H, $R_3 = R'_3 =$ Ph, $Q' = (CONH(CH_2)_6CONH)$ (Compound 441)</u>

20 mg of (1R, 5S, 7R)-5-(4-Nitro-phenyl)-3-phenyl-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-7-carboxylic acid methyl ester of formula (I) (Compound 31) (0.054 mmol) were added to 125.5 mg (1.08 mmol, 20 eq) of 1,6-diamino-hexane and the mixture heated at 65°C overnight. The crude is purified by chromatography (CH_2Cl_2-MeOH, 20:1 + NEt_3 1%), thus obtaining 8 mg (0.018 mmol, 34 %) of a yellow solid corresponding to (1R, 5S, 7R)-5-(4-nitro-phenyl)-3-phenyl-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-7-(6-amino-hexyl)-amide, i.e. the compound of formula (I) where $X = O$, R_1 p-NO_2Ph, $R_2 = H$, $R_3 =$ Ph, $R_6 = CONH(CH_2)_6NH_2$ (Compound 189) ($R_f = 0.32$) and 4 mg (0.0051 mmol, 10 %) of an orange solid corresponding to the dimeric compound of formula (III) of the title ($Rf = 0.67$).

- Compound 189: ^{1}H NMR ($CDCl_3$, δ): 832 (d, 2 H, $J = 8.4$ Hz), 7.83 (d, 2 H, $J = 8.8$ Hz), 7.30-7.22 (m, 2 H), 6.90-6.79 (m, 3 H), 6.25 (m, 1 H), 5.05 (s, 1 H), 4.74 (s, 1 H), 3.81-3.70 (m, 2 H), 3.28 (d, 1 H, $J = 9.8$ Hz,), 3.20-3.10 (m, 2 H), 2.92 (d, 1 H, $J = 11.6$ Hz), 2.61 (m, 2H), 1.78-1.15 (m, 10 H).
- dimeric compound of formula (III) of the title: ^{1}H NMR ($CDCl_3$, δ): 88(d, 4 H, $J = 8.8$ Hz), 7.82 (d, 4 H, $J = 10$ Hz), 7.31-7.24 (m, 4 H), 6.91-6.80 (m, 6 H), 6.25 (m, 2 H), 5.05 (s, 2 H), 4.75 (s, 2 H), 3.81-3.71 (m, 4 H), 3.29 (d, 2 H, $J = 11.6$ Hz,), 3.20-3.10 (m, 4 H), 2.92 (d, 2 H, $J = 11.6$ Hz), 1.54 (m, 4 H), 1.23 (m, 4 H).

BIOLOGICAL ACTIVITY

The biological activity of 3-aza-bicyclo(3.2.1)octanes of formula (I) and their dimeric forms of formula (II) and (III) was evaluated in different assays: induction of survival of PC12 cells in serum-free conditions, induction of proliferative activity

in PC3 prostatic carcinoma cell line, induction of VGF polypeptide synthesis, displacement of 125I-NGF binding to specific surface receptor, and induction of Trk-A autophosphorylation. In all of these assays human recombinant (hr)NGF was used as internal standard.

Effect of compounds on PC12 cell survival in serum-free conditions.

The biological activity of 3-aza-bicyclo(3.2.1)octanes of formula (I) and their dimeric forms of formula (II) and (III) was tested as ability to induce the survival of PC12 cells in serum-free conditions by using hrNGF as internal standard.

PC12 cells were detached from tissue flasks with PBS-EDTA (physiological saline solution added with ethylendiaminotetraacetic acid) and washed once with PBS to avoid residual amounts of serum. The cells were then diluted in RPMI-1640 medium without phenol red supplemented with penicillin and streptomycin and cultured in 96 well plates at the final concentration of 5 x 10^3/well. Standard curve was performed by adding in triplicate cultures different concentrations of hrNGF, in the range between 1-25 ng/ml. The compounds were instead added, in triplicate, at the final concentrations of 1, 10, 100 μM. The cells were then cultured for 60 hours at 37°C in a humidified, 5% CO_2, atmosphere. Then 10 μl of (3-[4.5-dimethylthiazol-2yl]-2.5-diphenyltetrazolium bromide (MTT, 0.5 mg/ml in isopropanol) were added to each well and plates, protected from the light, were left at 37°C for 4 hours. At the end of incubation, 100 μl of 50% dimethylformamide (in 20% SDS, pH 7.4) were added to each well. Colorimetric reaction was detected with a 96 well plate reader by recording the absorbance at 570 nm. Results were expressed as survival induced by compounds/spontaneous survival •100

Figure 1 shows the results obtained with 10 μM of the most representative compounds and with 1 nM of hrNGF.

Effect of compounds on proliferative activity of PC3 cell line.

The ability of 3-aza-bicyclo(3.2.1)octanes of formula (I) and their dimeric forms of formula (II) and (III) with substitutions reported in Table 1-4 to induce proliferation of PC3 cell line, in serum-free conditions, was tested by using hrNGF as internal standard.

PC3 cells were cultured in triplicate in 24 well plates at the final concentration of 10^4 cells/ml (final volume of 500 μl) in RPMI 1640 medium in the presence or

absence of 1, 10, 100 μM of the compounds or of different concentration (between 1-25 ng/ml) of hrNGF as internal standard. Cells were incubated for 60 hours in humidified, 5% CO_2, atmosphere. At the end of incubation 0.5 μCi of ^{3}H-thymidine were added to each well for 8 hours. Cells were then washed 6 times with PBS, lysed with 0.1 % Triton-X100 in 0.1 M phosphate buffer, and the radioactivity was recorded in a β-scintillation counter. Results were expressed as ratio between ^{3}H-thymidine incorporation (mean ± SD) of stimulated cultures and ^{3}H-thymidine incorporation of non stimulated cultures. Figure 2 shows the results obtained with 10 μM of selected compounds or with 1 nM hrNGF as internal standard.

Induction of VGF production by PC12 cells

The ability of 3-aza-bicyclo(3.2.1)octanes of formula (I) and their dimeric forms of formula (II) and (III) with substitution reported in Table 1-4 was tested also as ability to induce VGF production by PC12 cells. 5 x 10^6 PC12 cells were cultured in the presence or absence of 1, 10, 100 μM of the compounds or of 4 nM hrNGF as internal standard for 24 hours in humidified, 5% CO_2, atmosphere. Cells were lysed in 0.25% NP-40 in PBS supplemented with 1 mM PMSF (phenyl-methyl) and 1 mM leupeptin and protein concentration was measured in each sample by Bradford assay. Equal amounts of proteins (30 μg) were loaded in 8% SDS-polyacrilamide gel, electrophoresed, blotted onto nitrocellulose membrane and stained with monoclonal antibodies anti-VGF followed by peroxidase-conjugated anti-mouse IgG. Reaction was visualized by Enhanced Chemiluminiscent Reagent (ECL, Amersham) following the manifacturer instruction.

Figure 3 shows the results obtained with 10 μM of the selected (n. 91, 9, 323, 270) compounds or with 10 nM hrNGF. VGF is induced by the selected compounds as well as by hrNGF.

Displacement of ^{125}I-NGF binding to PC12 cells

The ability of selected compounds to displace the binding of NGF to specific surface receptor was evaluated through the classic binding techniques of iodinated ligand.

PC12 cells were detached from tissue flasks with PBS-EDTA, washed with HKR medium (10 mM Hepes, 125 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 1g/l glucose, 1g/l BSA) and incubated in triplicate in

HKR medium with 0.1 nM ^{125}I-NGF in the presence or absence of variable concentrations of the compounds to be assayed or of hrNGF as internal standard. Displacement curve was obtained by analyzing the resultant cell bound radioactivity in the presence of the compounds or of hrNGF with adequate software (Graphit 4).

Figure 4 a shows the displacement curve obtained with the compound n.9 used as competitor The analysis of data revealed a Kd of 165 nM ± 0.05. Figure 4b shows the displacement curve obtained by using hrNGF as competitor. The analysis of data revealed a Kd of 114 pM ± 0.01 as already reported.

Trk-A autophosphorylation

To evaluate the ability of the compounds 3-aza-bicyclo(3.2.1)octanes of formula (I) and their dimeric forms of formula (II) and (III) reported in Table 1-4 to induce Trk-A autophosphorylation, PC12 cells were cultured in medium supplemented with 5% FBS for 48 hours, washed and equilibrated in serum-free medium for 2 hours. 2.5 x 10^6 cells were then stimulated with 10 μM of selected compounds for 30 min or with 10 nM hrNGF as positive control. Cells were then lysed with 0.5% Triton-X100 in PBS supplemented with protease inhibitors (PMSF, aprotinin, pepstatin, leupeptin) and phosphatase inhibitors. Protein concentrations in each sample was evaluated by Bradford assay and equal amounts (50 μg) of proteins were loaded onto SDS-polyacrilamide gel, electrophoresed and blotted onto nitrocellulose membrane. Membranes was sained with rabbit anti-(Tyr 490 and Tyr 674/675) phosphorylated Trk-A (Cell Signaling Technology) used at the final dilution of 1:1000. After washing, membranes were stained with HRP-conjugated anti-rabbit IgG and the reaction was visualised by using ECL reagents following manufacturing instructions.

Figure 5 shows the results obtained with the compounds 272, 325, 9, 91 and with hrNGF used as internal standard. The selected compounds are able to induce Trk-A autophosphorylation thus triggering the transduction of biological signals.

Synergic activity

The synergic activity of multiple combinations of 3-aza-bicyclo(3.2.1)octanes of formula (I) and their dimeric forms of formula (II) and (III) was evaluated in the PC12 survival assay in serum-free condition.

PC12 cells were seeded in 96 well plates at the concentration of 5 x 10^3/well and cultured in triplicate in the presence or absence of 5 μM of selected compounds or of multiple combination of the same compounds at the final concentration of 10 μM. 0.5 nM hrNGF was used as internal standard. After 60 hours at 37°C in a humidified, 5% CO_2, atmosphere, 10 μl of (3-[4.5-dimethylthiazol-2yl]-2.5-diphenyltetrazolium bromide (MTT, 0.5 mg/ml in isopropanol) were added to each well and plates, protected from the light, were left at 37°C for 4 hours. At the end of incubation, 100 μl of 50% dimethylformammide (in 20% SDS, pH 7,4) were added to each well. Colorimetric reaction was detected with a 96 well plate reader by recording the absorbance at 570 nm. Results were expressed as survival induced by compounds/spontaneous survival *100. Figure 6 shows as selected combinations of 2 compounds (91 and 325) induce survival activity higher than the addition of activities induced by the single compound.